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C2C CAA CKE CQS CQT CRP CYX C1314 C1341 C1440 C1562 C1651 C214 C215 C22Y C220 C226 C247 C25Y C250 C251 C252 C255 C256 C28X C30Y C33Y C34Y C340 C342 C351 C352 C355 C357 C36Y C364 C366 C367 C368 C37Y C373 C396 C462 C613 C614 C624 C625 C628 C635 C638 C65X C658 C665 C668 C672 C675 C678 C699 C761 C762 C768 C80Y C800 C802 A5B BJA B170 B180 B21X B21Y B23X B23Y B230 B24Y B244 B246 B36Y B360 B40Y B401 B41Y B411 B44Y B440 B441 B443 B445 B446 B45Y B451 B453 B48Y B481 B482 B484 B485 B50Y B500 B501 B502 B503 B504 B505 B51Y B510 B52Y B528 B54Y B541 B542 B544 B546 B55Y B551 B552 B556 B56Y B565 B566 B57Y B574 B576 B60Y B605 B606 B65Y B654 B656 B67Y B672 B674 U1S S1313

(56) Documents cited

EP 0267723 A2

(58) Field of search

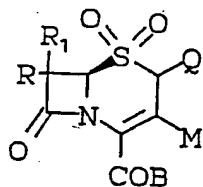
UK CL (Edition L) A5B, C2C CYX

INT CL⁶ A61K, C07D

Online databases: CAS ONLINE

(54) Substituted cephalosporin sulfone compositions useful in the treatment of leukemia

(57) Pharmaceutical compositions containing a compound of formula I



I

wherein the symbols are as defined in the specification; and epsilon-aminocaproic acid, heparin, trasyolol, prednisolone, cytosine arabinoside, 6-mercaptopurine, cytarabine, an anthracycline or a vitamin A derivative, may be used in the treatment of leukemia.

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TITLE OF THE INVENTION

SUBSTITUTED CEPHALOSPORIN SULFONES USEFUL IN THE
TREATMENT OF CANCER

BACKGROUND OF THE INVENTION

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This invention concerns the use of novel
azetidinones in the treatment of certain cancers
including promyelocytic leukemia, acute myelogenous
leukemia (FAB M1 and FAB M2), acute promyelocytic
leukemia (FAB M3), acute myelomonocytic leukemia (FAB
M5), erythroleukemia, chronic myelogenous leukemia,
chronic myelomonocytic leukemia, chronic monocytic
leukemia and conditions associated with leukemia
involving activity of PMN neutral proteases e.g.
disseminated intravascular coagulation. We have
found that the substituted azetidinones disclosed
herein are potent inhibitors of proteinase 3 (PR-3)
also known as myeloblastin.

See C. Labbaye, et al., Proc. Natl. Acad. Sci. USA, vol. 88, 9253-9256, (1991), Wegner autoantigen and myeloblastin are encoded by a single mRNA; D. Campanelli, et al., J. Exp. Med., vol. 172, 1709-1714, (1990), Cloning of cDNA for proteinase 3: A serine protease, antibiotic, and autoantigen from human neutrophils; and Bories, et al., Cell vol. 59, 959-968, (1989) Down-regulation of a serine protease, myeloblastin, causes growth arrest and differentiation of promyelocytic leukemia cells.

Recently, down regulation of PR-3 has been implicated in the proliferation and maintenance of a differentiated state of certain leukemia cells. In particular, Bories, et al., have shown that expression of this enzyme, hereinafter designated proteinase 3/myeloblastin, can be inhibited by treatment of HL-60 human leukemia cells with an antisense oligodeoxynucleotide and that such treatment induces differentiation and inhibits proliferation of these cells. Moreover, we have now demonstrated that the treatment of the HL-60 cell human leukemia cell line, among others, with the compounds of the instant invention, likewise results in the inhibition of proliferation and induction of differentiation in such cells.

Accordingly, we believe that treatment of leukemia such as promyelocytic leukemia, acute myelogenous leukemia (FAB M1 and FAB M2), acute promyelocytic leukemia (FAB M3), acute myelomonocytic leukemia (FAB M4), acute monocytic leukemia (FAB M5), erythroleukemia, chronic myelogenous leukemia, chronic myelomonocytic leukemia, chronic monocytic

leukemia and conditions associated with leukemia involving activity of PMN neutral proteases e.g. disseminated intravascular coagulation, comprising: administration of a therapeutically effective amount of compound of formula I will result in remission of the disease state. Administration may be either oral or parenteral.

We have also found that a group of new substituted azetidinones are potent inhibitors of PMN elastase and PR3 and therefore are useful anti-inflammatory and antidegenerative agents.

Proteases from granulocytes and macrophages have been reported to be responsible for the chronic tissue destruction mechanisms associated with inflammation, including rheumatoid arthritis and emphysema. Accordingly, specific and selective inhibitors of these proteases are candidates for potent anti-inflammatory agents useful in the treatment of inflammatory conditions resulting in connective tissue destruction, e.g. rheumatoid arthritis, emphysema, bronchial inflammation, osteoarthritis, spondylitis, lupus, psoriasis and acute respiratory distress syndrome.

The role of proteases from granulocytes, leukocytes or macrophages are related to a rapid series of events which occurs during the progression of an inflammatory condition:

- (1) There is a rapid production of prostaglandins (PG) and related compounds synthesized from arachidonic acid. This PG

synthesis has been shown to be inhibited by aspirin-related nonsteroidal anti-inflammatory agents including indomethacin and phenylbutazone. There is some evidence that protease inhibitors prevent PG production;

(2) There is also a change in vascular permeability which causes a leakage of fluid into the inflamed site and the resulting edema is generally used as a marker for measuring the degree of inflammation. This process has been found to be induced by the proteolytic or peptide cleaving activity of proteases, especially those contained in the granulocyte, and thereby can be inhibited by various synthetic protease inhibitors, for example, N-acyl benzisothiazolones and the respective 1,1-dioxides. Morris Zimmerman et al., J. Biol. Chem., 255, 9848 (1980); and

(3) There is an appearance and/or presence of lymphoid cells, especially macrophages and polymorphonuclear leukocytes (PMN). It has been known that a variety of proteases are released from the macrophages and PMN, further indicating that the proteases do play an important role in inflammation.

In general, proteases are an important family of enzymes within the peptide bond cleaving enzymes whose members are essential to a variety of

normal biological activities, such as digestion, formation and dissolution of blood clots, the formation of active forms of hormones, the immune reaction to foreign cells and organisms, etc., and in pathological conditions such as the degradation of structural proteins at the articular cartilage/pannus junction in rheumatoid arthritis etc.

Elastase is one of the proteases. It is an enzyme capable of hydrolyzing the connective tissue component elastin, a property not contained by the bulk of the proteases present in mammals. It acts on a protein's nonterminal bonds which are adjacent to an aliphatic amino acid. Neutrophil elastase is of particular interest because it has the broadest spectrum of activity against natural connective tissue substrates. In particular, the elastase of the granulocyte is important because, as described above, granulocytes participate in acute inflammation and in acute exacerbation of chronic forms of inflammation which characterize many clinically important inflammatory diseases.

Proteases may be inactivated by inhibitors which block the active site of the enzyme by binding tightly thereto. Naturally occurring protease inhibitors form part of the control or defense mechanisms that are crucial to the well-being of an organism. Without these control mechanisms, the proteases would destroy any protein within reach. The naturally occurring enzyme inhibitors have been shown to have appropriate configurations which allow them to bind tightly to the enzyme. This configuration is part of the reason that inhibitors bind to

the enzyme so tightly (see Stroud, "A Family of Protein-Cutting Proteins" Sci. Am. July 1974, pp. 74-88). For example, one of the natural inhibitors, α_1 -Antitrypsin, is a glycoprotein contained in human serum that has a wide inhibitory spectrum covering, among other enzymes, elastase both from the pancreas and the PMN. This inhibitor is hydrolyzed by the proteases to form a stable acyl enzyme in which the active site is no longer available. Marked reduction in serum α_1 -antitrypsin, either genetic or due to oxidants, has been associated with pulmonary emphysema which is a disease characterized by a progressive loss of lung elasticity and resulting respiratory difficulty. It has been reported that this loss of lung elasticity is caused by the progressive, uncontrolled proteolysis or destruction of the structure of lung tissue by proteases such as elastase released from leukocytes. J. C. Powers, TIBS, 211 (1976).

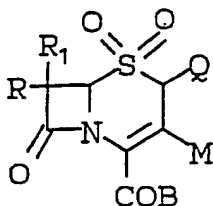
Rheumatoid arthritis is characterized by a progressive destruction of articular cartilage both on the free surface bordering the joint space and at the erosion front built up by synovial tissue toward the cartilage. This destruction process, in turn, is attributed to the protein-cutting enzyme elastase which is a neutral protease present in human granulocytes. This conclusion has been supported by the following observations:

(1) Recent histochemical investigations showed the accumulation of granulocytes at the cartilage/pannus junction in rheumatoid arthritis; and

5 (2) a recent investigation of mechanical behavior of cartilage in response to attack by purified elastase demonstrated the direct participation of granulocyte enzymes, especially elastase, in rheumatoid cartilage
10 destruction. H. Menninger et al., in Biological Functions of Proteinases, H. Holzer and H. Tschesche, eds. Springer-Verlag, Berlin, Heidelberg, New
15 York, pp. 196-206, 1979.

SUMMARY OF THE INVENTION

The present invention is directed to the treatment of leukemia, such as promyelocytic
20 leukemia, acute myelogenous leukemia (FAB M1 and FAB M2), acute promyelocytic leukemia (FAB M3), acute myelomonocytic leukemia (FAB M4), acute monocytic leukemia (FAB M5), erythroleukemia, chronic
25 myelogenous leukemia, chronic myelomonocytic leukemia, chronic monocytic leukemia and conditions associated with leukemia involving activity of PMN neutral proteases e.g. disseminated intravascular
30 coagulation with compounds of formula I.



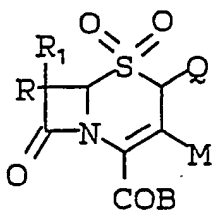
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or a pharmaceutically acceptable salt thereof.

Treatment of leukemia cells comprising:
administration of a therapeutically effective amount
of a compound of formula I results in the inhibition
of proteinase 3/myeloblastin, inhibition of elastase,
inhibition of proliferation of the leukemia cells,
induction of differentiation of the leukemia cells,
and remission of the disease state.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment the invention concerns a
method of treating leukemia comprising:
administration to a patient in need of such treatment
of a therapeutically effective amount of compound of
formula I



I

or a pharmaceutically acceptable salt thereof,
wherein R is hydrogen, chlorine or fluorine

M is:

- (1) trifluoromethyl;
- (2) chloro or fluoro;
- (3) -COOH;
- (4) -CHO; or
- (5) -CH₂A wherein A represents
 - (a) hydrogen;
 - (b) halo;
 - (c) hydroxy;
 - (d) alkoxy;
 - (e) aryloxy;
 - (f) aralkyloxy;
 - (g) unsubstituted or substituted mercapto;
 - (h) acylthio;
 - (i) acyloxy especially alkanoyloxy or aryl-carbonyloxy such as acetoxy, benzyloxycarbonyloxy, benzoyloxy; and succinoyloxy; substituted or unsubstituted carbamoyl, thiocarbamoyl and N-alkyl or N,N-dialkyl derivatives thereof;
 - (j) a quaternary ammonium group, for example, $-\text{NH}_3^+$, $-\text{NHE}^2$, or $-\text{NE}^3$ where E represents loweralkyl, aryl or aralkyl;
 - (k) unsubstituted or substituted amino or amido group especially -NH₂, -CONH₂ and N-alkyl or N,N-dialkyl derivatives thereof;

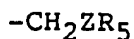
(1) R-SO- wherein R is C₁₋₁₆alkyl or C₆₋₁₀aryl; or

(m) R-SO²-;

(6) -CH=CHR.

Thus, CH₂A can be a halomethyl such as chloromethyl, bromomethyl or fluoromethyl.

When CH₂A is a substituted hydroxy or substituted mercapto group, it can be shown by the formula

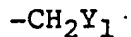


where Z is oxygen or sulfur, and R₅ is an acyl group; a straight chain or branched chain loweralkyl, alkenyl or alkynyl group; an aryl group; an aralkyl group; or a heterocyclic group such as heteroaryl, heterocycloalkyl e.g., 1,3-dioxacyclohex-4-yl, piperidino, morpholino, oxacyclopropyl, pyrrolidino, tetrazolo, benzothiazolo, imidazolidino, pyrazolidino, and piperazino; or heterocycloalkenyl such as pyrrolino, 2-imidazolino, 3-pyrazolino or isoindolino. These groups can be unsubstituted or can be substituted by radicals such as alkyl, alkoxy, halo, cyano, carboxy, carbamoyl, azido,, sulfo, amino,, substituted amino, haloalkyl, carboxyalkyl, carbamoylalkyl, N-substituted carbamoylalkyl, guanidino, N-substituted guanidino, guanidoalkyl, sulfamyl, substituted sulfamyl, and the like. Representative of the CH₂A groups are methoxymethyl, n-propoxymethyl, methylthiomethyl, acetoxymethyl, propionyloxymethyl, benzoyloxymethyl, (p-chlorobenzoyl)oxymethyl, succinoyloxymethyl, (p-methylbenzoyl)oxymethyl, pivaloyloxymethyl,

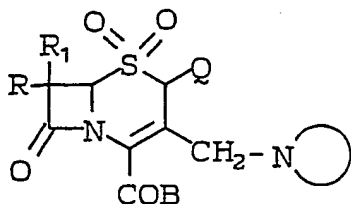
(1-adamantyl)-carboxymethyl, butanoyloxymethyl, carbamoyloxymethyl, (N-methylcarbamoyl)oxymethyl, (N-ethylcarbamoyl)oxymethyl, [N-(2-chloroethyl)carbamoyl]oxymethyl, (N-phenylcarbamoyl)oxymethyl, [N-(carboxymethyl)-carbamoyl]oxymethyl, (N-p-sulfophenyl-carbamoyl)oxymethyl, p-carboxymethylphenyl-carbamoyloxymethyl, methoxycarbonyloxymethyl, isobutanoyloxymethyl, cyclobutyl-carbonyloxymethyl, carbamoylthiomethyl, (ethoxythiocarbonyl)thiomethyl, (n-propoxythiocarbonyl)thiomethyl, (cyclopentanoxythiocarbonyl)thiomethyl, methylthiomethyl, N,N-diethylthiocarbamoylthiomethyl, N-methylpiperazinium-1-thiocarbonylthiomethyl, N,N-dimethylpiperaziny-1-thiocarbonylthiomethyl, 2-furoylthiomethyl, isothiuroniummethyl, (5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl, p-tolylsulfonylthiomethyl, 2-benzothiazolothiomethyl, mesyloxymethyl, 1-methyl-1,2,3,4-tetrazolyl-5-thiomethyl, tosyloxymethyl, sulfamoyloxymethyl, 1-naphthoyloxymethyl, 2-furylacetoxy-methyl, cinnamoyloxymethyl, p-hydroxycinnamoyloxymethyl, p-sulfo-cinnamoyloxymethyl and 1R:2S-epoxypropylphosphonyloxymethyl.

Alternatively, when CH₂A is hydroxymethyl, the cephalosporin can also exist as the lactone which is formed by internal esterification with the adjacent carboxy group.

The substituent CH_2A can also be a group of the general formula



wherein Y_1 represents amino or substituted amino including nitrogen heterocycles and substituted heterocyclic groups as described for R_5 . Y_1 may also be nitrogen which is part of the heterocyclic system as shown below.



Examples of such groups that might be mentioned are aminomethyl, acetamidomethyl, carbamoylaminoethyl, N,N -dimethylaminomethyl, N -(2-chloroethyl)-aminomethyl, 5-cyano-triazol-1-yl-methyl, 4-methoxy-carbonyltriazol-1-yl-methyl.

When A is amino the cephalosporin compound can also exist as the lactam formed by loss of water with the adjacent carboxy group.

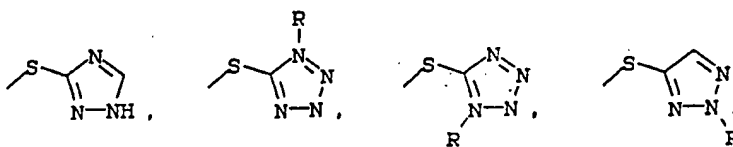
Representative of the quaternary ammonium groups representing A that might be mentioned are pyridinium, 3-methylpyridinium, 4-methylpyridinium, 3-chloropyridinium, 3-bromopyridinium, 3-iodopyridinium, 4-carbamoylpyridinium, 4-(N -hydroxymethyl-carbamoyl)pyridinium, 4-(N -carbomethoxycarbamoyl)-pyridinium, 4-(N -cyanocarbamoyl)pyridinium,

4-carboxymethylpyridinium, 4-hydroxymethylpyridinium, 4-trifluoromethylpyridinium, quinolinium, picolinium and lutidinium.

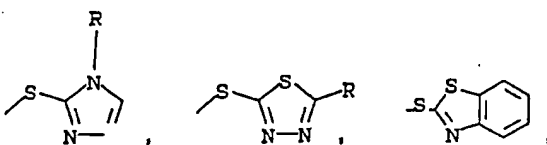
When A is mercapto, it may be -SH, $-\text{S}-\overset{\text{O}}{\underset{\text{S}}{\text{C}}}-\text{O}-$

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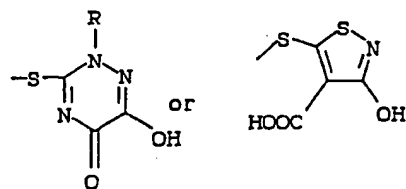
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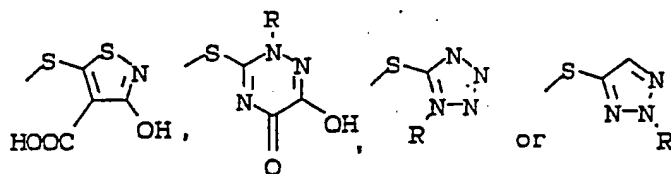
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alkyl, alkylthio, arylthio, aralkylthio or heterocyclothio, wherein R represents C_{1-16} loweralkyl, C_{6-10} aryl or CH_2COOH .

The class representing A are (a) hydrogen; (b) halo; (c) hydroxy; (d) alkoxy; (e) aryloxy; (f) aralkyloxy; (g) substituted or unsubstituted mercapto especially $-\text{S}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{O}-\text{R}$,

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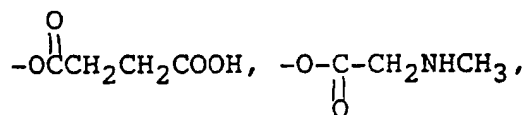
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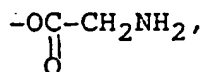
(h) acylthio; or (i) acyloxy. The acyl group can be
 10 a loweralkanoyl group of 2-6 carbon atoms such as
 acetyl, $-\text{COC}_2\text{H}_5$ or $-\text{COC}_3\text{H}_7$, carbamoyl, or
 thiocarbamoyl and N-alkyl or N,N-dialkyl derivatives
 thereof. The alkyl group of the foregoing substi-
 15 tuted by radicals such as alkoxy, halo, amino,
 cyano, carboxy, sulfo, and the like.

A subclass representing, A is O
 (a) alkanoyloxy especially $-\text{OCCH}_3$

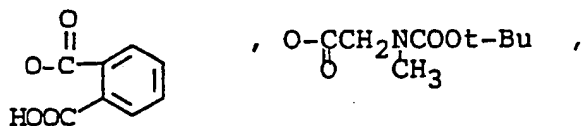
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The substituent R_1 in formula (I) above is

- (a) hydrogen;
- (b) hydroxy;
- (c) mercapto;
- (d) substituted oxy;
- (e) substituted thio;
- (f) hydrocarbyl or substituted hydrocarbyl group;
- (g) cyano;
- (h) carbonyl or thiocarbonyl containing substituents bonded by said carbonyl or thiocarbonyl radical;
- (i) halo;
- (j) phosphono or a substituted phosphono group;

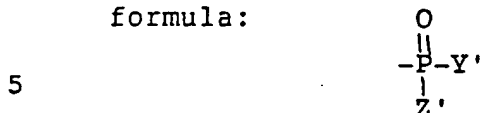
The oxy or thio substituent represented by R_1 in formula (I) can be a substituted hydroxy or mercapto group such as $-XR'_1$ wherein X is oxygen or sulfur and R'_1 is a hydrocarbyl group, preferably a straight or branched lower alkyl group of 1-6 carbon atoms, a straight or branched chain loweralkenyl or loweralkynyl group of 3-6 carbon atoms, a monocyclic aryl group such as phenyl, furyl, pyrrol and pyridyl, or an aralkyl group such as benzyl. These alkyl, alkenyl, alkynyl, aryl or aralkyl groups can be substituted with groups such as hydroxy, halo, nitro, amino, carboxy, thio, and the like. Other specific substituents represented by R_1 that might be mentioned are groups of the formula $-OAc$, $-SAc$, $-SO_3H$, $-SO_2NH_2$, $-SO_2R_2$, $-SO_2NR_3R_4$, $-OCOOR_2$, $-SOR_2$, $-OCOSR_2$, $-OCONR_3R_4$, and the like wherein Ac represents an acyl group such as a formyl or lower-

alkanoyl, R_3 and R_4 represent hydrogen, lower-alkyl, acyl and loweralkoxy, and R_2 represents loweralkyl, haloloweralkyl, aryl, aralkyl and substituted derivatives of such groups.

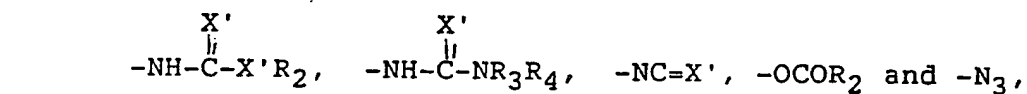
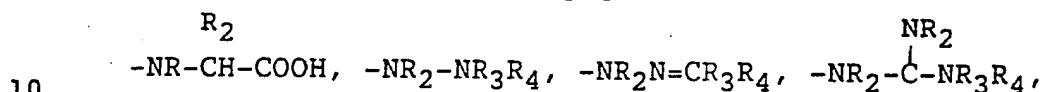
5 When R_1 is hydrocarbyl it can be straight or branched loweralkyl, straight or branched lower-alkenyl, loweralkynyl, aralkyl, cycloalkyl, a monocyclic aryl group, or a monocyclic heterocyclic group which can also be substituted with one or more
10 groups such as halo, hydroxy, alkoxy, amino, nitro, sulfonyl, sulfamoyl, acyloxy, carbamoyloxy, carboxy, carboxamido and N-substituted carboxamido. Representative examples of such groups are C_{1-6} alkyl such as methyl, trifluoromethyl, ethyl, n-propyl, isopropyl, t-butyl; C_{2-6} alkenyl especially allyl,
15 α -butenyl; C_{2-6} alkynyl such as ethynyl and methylethynyl; loweraralkyl such as benzyl, p-methoxybenzyl, phenethyl; phenyl, p-aminophenyl; cyclopropyl, cyclopentyl and 4-hydroxycyclohexyl;

20 R_1 in formula (I) above may also represent
cyano or a group of the general formula $\begin{array}{c} X' \\ || \\ -C-R'' \end{array}$
wherein X' is oxygen or sulfur, and R'' is hydrogen, halo, hydroxy, mercapto, amino, substituted amino,
25 alkyl, aryl, aralkyl, aralkoxy such as benzyloxy, alkoxy or aryloxy such as phenoxy, pyrroloxy, furyloxy, and thienyloxy, alkylthio or arylthio. Examples of these substituents are $-COOH$, $-CSSH$, $-COR_2$,
30 $-COOR_2$, $-COSR_2$, $-CSSR_2$, $-CONH_2$, $-CSNH_2$, $-CSR_2$, $-CONHR_2$, $-CSNH$, $-CONR_3R_4$ and $-CSNR_3R_4$ wherein R_2 represents a straight or branched chain alkyl group of 1-6 carbon atoms and R_3 and R_4 represent hydrogen or R_2 ;

Finally, the substituent R_1 in formula (I) represents phosphono or a metal or ammonium salt thereof, or a substituted phosphono group of the formula:



where Y' and Z' are the same or different and represent $-\text{OR}_2$, $-\text{NR}_3\text{R}_4$,



where R_2 represents hydrogen or a hydrocarbyl radical, R_3 and R_4 represent hydrogen, hydrocarbyl, alkoxy or an acyl radical, and X' represents oxygen or sulfur.

In one class, R_1 is

- (1) hydroxy;
- (2) OR_1 , where R_1 represents hydrocarbyl group;
- (3) C_{1-6} alkylthio;
- (4) C_{1-6} alkylsulfinyl;
- (5) C_{1-6} alkylsulfonyl;
- (6) halo such as fluoro, chloro, bromo or iodo;
or;
- (7) hydrogen; or
- (9) C_{1-6} alkyl.


In one subclass, R_1 is

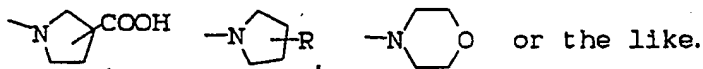
- (1) C_{1-3} alkyl;
 - (2) hydroxy;
 - (3) OR'_1 where R'_1 is
 - (a) C_{1-6} alkyl especially methyl, ethyl, n-propyl;
 - (b) $-C_6H_5$;
 - (c) $-CH_2CH_2C_6H_5$; or
 - (d) $\begin{array}{c} O \\ || \\ -C-R \end{array}$ where R represents hydrogen, C_{1-6} alkyl, phenyl, substituted or unsubstituted benzyl, or C_{1-6} alkylamino such as CH_3NH- , C_2H_5NH- ;
 - (4) halo especially Cl or F; or
 - (5) $-SO_2R$.
- B of Formula (I) above represents OB_1 , or NB_2B_3 wherein B_1 and B_2 independently are:
- (a) straight or branched chain alkyl having from 1 to 20 carbon atoms, ethyl, isopropyl, t-butyl, pentyl or hexyl;
 - (b) aryl having from 6 to 10 carbon atoms;
 - (c) cycloalkyl having from 3 to 8 carbon atoms;
 - (d) alkenyl having from 2 to 20 carbon atoms;
 - (e) cycloalkenyl having from 5 to 8 carbon atoms;
 - (f) alkynyl having from 2 to 20 carbon atoms;
 - (g) alkoxy having from 1 to 10 carbon atoms;
 - (h) aralkyl, alkaryl, aralkenyl, aralkynyl, alkenylaryl or alkynylaryl wherein alkyl, aryl, alkenyl and alkynyl are as previously defined;
 - (i) loweralkenylalkyl;
 - (j) alkanoylalkyl;

- (k) alkanoyloxyalkyl;
- (l) alkoxyalkyl;
- (m) alkanoyloxy;
- (n) a heterocyclic group including heterocyclic alkyl or heterocyclic alkenyl.

5 The above groups (a)-(n) can be unsubstituted or can be substituted by radicals such as alkyl, hydroxy, alkoxy, halo, nitro, mercapto, amino, substituted amino, cyano, carboxy, sulfoamino, carbamoyl, carbamoyloxy, sulfonyl, sulfinyl, sulfamoyl, azido, 10 amino, substituted amino, carboxamido or N-substituted carboxamido;

B_3 is hydrogen or B_1 ; and

B_2 and B_3 may join together and form part of the heterocyclic group -N  e.g. :



Representative examples of such groups are C_{1-6} alkyl especially methyl, ethyl or t-butyl, allyl, 3-butenyl, methoxyethyl, benzyl, 25 p-carbomethoxybenzyl, m-carbomethoxybenzyl, p-sulfonylbenzyl, m-fluorobenzyl, o,p-dinitrobenzyl, o,p-dichlorobenzyl, p-methylbenzyl, m-methoxybenzyl, o-methylthiobenzyl, benzhydryl, $-CH_2COOH$, $-CH_2COOt-Bu$, 30 $CH_2CH_2CH_2COOCH_3$, $-CH_2COOC_2H_5$, and the like.

In one class, B_1 and B_2 independently are substituted or unsubstituted

- (1) aralkyl;
- (2) aryl;
- 5 (3) straight or branched loweralkyl;
- (4) straight or branched loweralkenyl;
- (5) cycloalkyl;
- (6) alkanoyloxyloweralkyl;
- (7) alkanoylloweralkyl;
- 10 (8) alkoxyloweralkyl; or
- (9) haloalkyl;

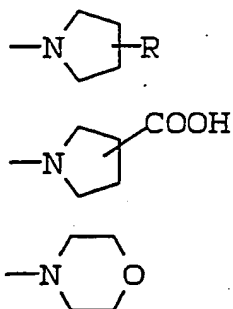
B_3 is hydrogen or B_1 ; and
 B_2 and B_3 may join together and form part of the heterocyclic group as defined previously;

- 15 Even more preferably, B_1 and B_2 independently are substituted or unsubstituted

- (1) benzyl;
- (2) methyl;
- (3) t-butyl;
- 20 (4) $-\text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2$ or $\text{CH}_2-\text{CH}=\text{C}(\text{CH}_3)_2$;
- (5) $-\text{CH}_2\text{COOH}$;
- (6) alkanoyloxymethyl; or
- (7) alkanoylmethyl;

B_3 is hydrogen or B_1 ; and

- 25 B_2 and B_3 may join together and form part of the heterocyclic group selected from a group consisting of:



5

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Q in formula (I) represents

15

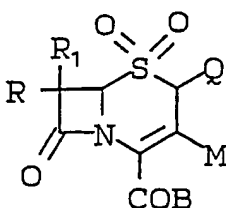
- (1) hydrogen;
- (2) C₁₋₆ alkyl especially methyl, ethyl, isopropyl, n-pentyl or n-hexyl;
- (3) halo C₁₋₆alkyl especially chloro or fluoro C₁₋₆alkyl; or
- (4) hydroxy C₁₋₆alkyl;
- (5) methylene or substituted methylene especially C₁₋₆ alkylmethylene, unsubstituted or substituted phenylmethylene phenylthiomethylene, phenylsulfinylmethylene or phenyl sulfonylmethylene;
- (6) C₁₋₆alkoxy C₁₋₆ alkyl;
- (7) aralkyl especially unsubstituted or substituted benzyl or phenethyl;
- (8) unsubstituted or substituted phenylthio C₁₋₆alkyl, phenylsulfonyl C₁₋₆;
- (9) unsubstituted or substituted phenoxy C₁₋₆alkyl; or
- (10) unsubstituted or substituted phenylamino C₁₋₆alkyl.

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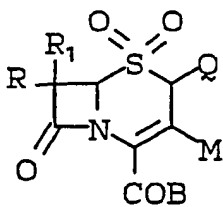
In a third embodiment the invention concerns a method of inhibiting proteinase 3/myeloblastin and elastase, comprising:
administration to a patient in need of such inhibition of a therapeutically effective amount of compound of formula I



I

or a pharmaceutically acceptable salt thereof as defined in this specification.

In a fourth embodiment the invention concerns a method of inducing cell differentiation in leukemia cells comprising:
administration to a patient in need of such induction of a therapeutically effective amount of compound of formula I

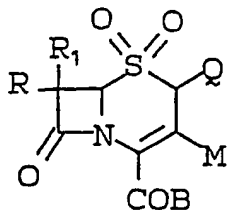


I

or a pharmaceutically acceptable salt thereof as defined in this specification.

We also find that with regard to each of the above embodiments, co-administration of a compound of formula I as defined above, with an agent or agents known in the art for treatment of leukemia, including, but not limited to epsilon-aminocaproic acid, heparin, trasylol (aprotinin); prednisolone; cytosine arabinoside; b-mercaptapurine; cytarabine; an anthracycline (see Young et. al. (1981) N. Engl. J. Med. 305:139) such as dauorubicin, doxorubicin and epidoxorubicin; Vitamin A derivatives including retinoids and all-trans-retinoic acid (See Ellison R.R. et.al. (1968) Blood 32:507, Arabinosyl Cytosine: A useful agent in the treatment of leukemia in adults; Cytarabine: Therapeutic new dimensions, Semin. Oncol. 12:1 (1985, supp 3); Weinstein H.J. et al (1983) Blood 62:315, Chemotherapy for acute myelogenous leukemia in children and adults) results in an enhanced therapeutic response.

Accordingly, in a fifth embodiment the invention concerns a pharmaceutical composition comprising:
a pharmaceutical carrier, a therapeutically effective amount of compound selected from the group consisting of epsilon-aminocaproic acid, heparin, trasylol, prednisolone, cytosine arabinoside, b-mercaptapurine, cytarabine, an anthracycline and a vitamin A derivative; and a therapeutically effective amount of compound of formula I

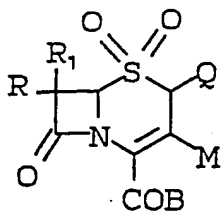


I

or a pharmaceutically acceptable salt thereof
as defined in this specification.

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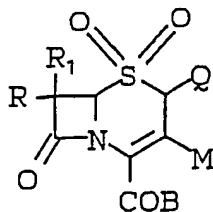
In a sixth embodiment the invention concerns
a method of treating leukemia comprising:
co-administration to a patient in need of such
treatment of a therapeutically effective amount of
15 compound selected from the group consisting of
epsilon-aminocaproic acid, heparin, trasylol,
prednisolone, cytosine arabinoside, b-mercaptapurine,
cytarabine, an anthracycline, and a vitamin A
derivative; and a therapeutically effective amount of
20 compound of formula I.



I

or a pharmaceutically acceptable salt thereof
30 as defined in this specification.

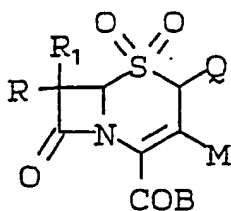
In a seventh embodiment the invention concerns a method of inhibiting proteinase 3/myeloblastin, comprising:
co-administration to a patient in need of such inhibition of a therapeutically effective amount of
5 compound selected from the group consisting of epsilon-aminocaproic acid, heparin, trasyolol, prednisolone, cytosine arabinoside, b-mercaptapurine, cytarabine, an anthracycline, and a vitamin A
10 derivative; and a therapeutically effective amount of compound of formula I



I

or a pharmaceutically acceptable salt thereof
20 as defined in this specification.

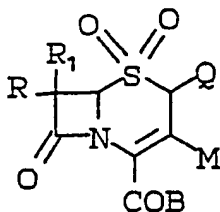
In an eighth embodiment the invention concerns a method of inhibiting proteinase 3/myeloblastin and elastase, comprising:
25 administration to a patient in need of such inhibition of a therapeutically effective amount of compound selected from the group consisting of epsilon-aminocaproic acid, heparin, trasyolol, prednisolone, cytosine arabinoside, b-mercaptapurine,
30 cytarabine, an anthracycline, and a vitamin A derivative; and a therapeutically effective amount of compound of formula I



I

5
10 or a pharmaceutically acceptable salt thereof
as defined in this specification.

In a ninth embodiment the invention concerns
a method of inducing cell differentiation in leukemia
cells comprising:
15 administration to a patient in need of such inducing
of a therapeutically effective amount of compound
selected from the group consisting of
epsilon-aminocaproic acid, heparin, trasyolol,
prednisolone, cytosine arabinoside, b-mercaptapurine,
20 cytarabine, an anthracycline and a vitamin A
derivative; and a therapeutically effective amount of
compound of formula I



I

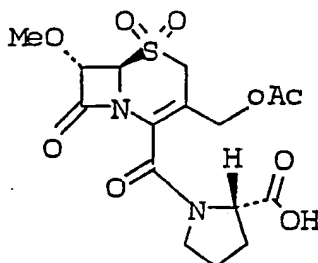
25
30 as defined in this specification.

It has been found that the compounds of formula I, inhibit proteinase 3/myeloblastin as shown in Tables 1a and 1b.

It has also been found that the compounds of Formula (I) have anti-inflammatory antidegeneration activity and are effective in the prevention and inhibition of edema and granuloma tissue formation as shown below in Table II by the effective inhibition of the proteolytic function of human granulocyte elastase.

TABLE 1a

SECOND ORDER RATE CONSTANTS FOR THE INHIBITION OF
HUMAN PROTEINASE 3



$$\text{SORC} = 2,700 \text{ M}^{-1}\text{SEC}^{-1}$$

ASSAY

The PR3 catalyzed hydrolysis of MeO-Succ-AAPV-pNA was measured in a spectrophotometer monitoring absorbance at 410 m,m. The enzymatic activity was determined in 45 mM TES at pH 7.5, 450 mM NaCl and 10 % DMSO. The

data were fit by non-linear regression to equation 1 to obtain the initial rates. The nonlinear progress curves observed with time dependent inhibitors were fit to equation 2 to obtain the first order rate constant K_{obs} .

$$\text{EQN 1} \quad Y = v_i X + B$$

$$\text{EQN 2} \quad Y = v_s * X + [(v_o - v_s) (1 - e^{(-K_o * x)}) \times K_o] + A_o$$

Kinetic constants for the inhibition of PR3 catalyzed hydrolysis of 0.2 mM MoO-succ-AAPV-pNA were determined by varying the concentration of inhibitor present in the reaction vessel.

ASSAY 1b

Human Proteinase 3 was purified from an acidic extract of human PMN granules using MATREX GEL ORANGE A chromatography followed by cation exchange on MONO-S FPLC. Purity was determined by N-terminal amino acid sequencing, amino acid analysis, silver staining of purified proteins separated by SDS-PAGE, western blotting using selective antisera to granule proteins, C_{18} reverse phase HPLC and kinetic analysis using selective inhibitors.

PR3 was assayed by mixing 4 picomoles purified enzyme in 200 μ l KPT high salt buffer (1M NaCl, 50 mM potassium phosphate, 0.075 % Tween 20) with 0.2 to 1000 picomoles inhibitor in 2 μ l DMSO. The mixture was incubated for 15-30 min. at 25 °C. Enzyme activity was determined after the addition of 50 μ l

O=C1NC(=O)C2(C1)S(=O)(=O)C(=C(C2)C)C(=O)C3=C(C=C(C3)C)C

I

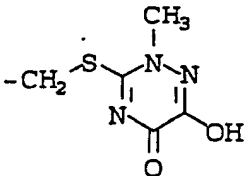
wherein R is H; Q is H and

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- 32 -

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| | R ₁ | M | B | pmoles/well | % inhibition |
|----|--------------------------------------|-------------------------------------|--------------------------------------|-------------|--------------|
| 5 | -O-SEC butyl | -CH ₂ OCOCH ₃ | -O-t-Bu | 2 | 18.5 ± 3.6 |
| | | | | 100 | 24.1 ± 7.4 |
| | | | | 1000 | 68.9 ± 3.1 |
| 10 | -OEt | -CH ₂ OCOCH ₃ | -OMe | 2 | 74.9 ± 4.7 |
| | | | | 100 | 93.7 ± 2.2 |
| | | | | 1000 | |
| 15 | -OCH ₂ CH ₂ Ph | -CH ₂ OCOCH ₃ | -OMe | 2 | 29.3 ± 3.9 |
| | | | | 100 | 91.9 ± 4.7 |
| | | | | 1000 | |
| 20 | -OMe | -CH ₂ OCOCH ₃ | -OCH ₂ - <i>p</i> -(o)OH) | 2 | 40.7 ± 3.3 |
| | | | | 100 | 99.3 ± 4.7 |
| | | | | 1000 | 100 ± 1.5 |
| 25 | | | | | |
| | | | | | |
| | | | | | |
| 30 | | | | | |
| | | | | | |
| | | | | | |

| | R ₁ | M | B | pmoles/well | % inhibition |
|----|----------------|---|-------------------------------|----------------|----------------|
| 5 | -OMe |  | -O-t-Bu | 2 10 100 | 30 85 90 |
| 10 | -OMe | -CH ₂ OCO-NHCH ₂ φ | -O-CH ₂ φ-(p-COOH) | 2 10 100 | 18 85 94 |
| | -OMe | -CH ₂ OCOφ | -O-t-Bu | 2 10 100 | 34 87 91 |
| 15 | -OMe | -CH ₂ OCONHφ | -N(Me)CH ₂ CO-t-Bu | 2 10 100 | 28 86 90 |

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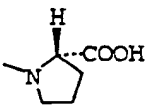

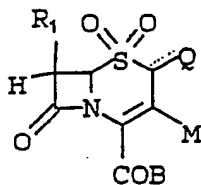
| R ₁ | M | B | pmoles/well | % inhibition |
|----------------|-------------------------------------|---|-------------|--------------|
| -OMe | -CH ₂ OCOCH ₃ |  | 2 | 52.0 |
| | | | 4 | 67.6 |
| | | | 10 | 86.0 |
| -OEt | -CH ₂ OCOCH ₃ |  | 20 | 95.2 |
| | | | 2 | 26.4 |
| | | | 4 | 86.0 |

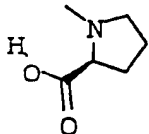
TABLE II

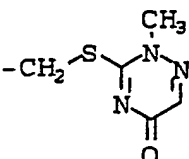
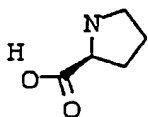


5

| | R ₁ | M | B | Q | ED ₅₀ * |
|----|-------------------|-------------------------------------|---|-----------------|--------------------|
| 10 | -OCH ₃ | -CH ₂ OCOCH ₃ | -OCH ₃ | H | 0.08 |
| | " | " | -OCH ₂ Ø | H | 0.03 |
| 15 | " | " | -OCH ₂ Ø-(p-COOCH ₃) | H | 0.02 |
| | " | " | -OCH ₂ COtBu | H | 0.02 |
| 20 | " | " | -OCH ₂ CH=C(CH ₃) ₂ | H | 1.0 |
| | " | " | -O(CH ₂) ₃ COOCH ₃ | H | 0.05 |
| | " | " | -NHCH ₂ COOtBu | H | 0.9 |
| 25 | " | " | -N(CH ₃) ₂ | H | 0.6 |
| | " | " | -OtBu | CH ₃ | 0.03 |
| 30 | " | " | -OtBu | CH ₂ | 0.03 |

µg/ml of compound

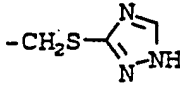
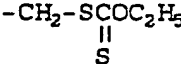
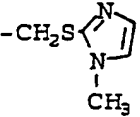
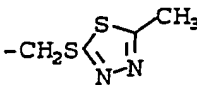
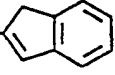
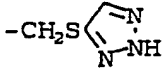
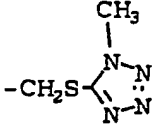
| | R ₁ | M | B | O | ED ₅₀ |
|----|--------------------------------|--|---|-----------------------------------|------------------|
| 5 | -OCH ₃ | -CH ₂ OCOCH ₃ | -Ot Bu | CH ₂ SO | 0.03 |
| | " | " | -Ot Bu | CH ₂ SO ₂ O | 0.02 |
| | " | " | -Ot Bu | CH ₂ O | 15.0 |
| | -C ₂ H ₅ | " | Ot Bu | H | 1.0 |
| | " | -CH ₂ OC(=O)NHCH(CH ₃)COOH | Ot Bu | H | 1.0 |
| 10 | " | -CH ₂ OCOCH ₂ CH ₂ COOH | Ot Bu | H | 0.8 |
| | " | -CH ₂ OCOCH ₃ | -OCH ₂ O | H | 0.4 |
| | " | -CH ₂ OCOCHO NH ₂ | -Ot Bu | H | 0.4 |
| | -OCH ₃ | -CH ₂ OCOCH ₃ |  | H | 5 |
| 15 | | | | | |
| 20 | | | | | |
| 25 | | | | | |
| 30 | | | | | |

| | R ₁ | M | B | O | IC ₅₀ |
|----|---------------------------------|---|--|---|------------------|
| 5 | " | " | -N(CH ₃)CH ₂ COOH | H | |
| | " |  | " | H | |
| 10 | " | " |  | H | |
| 15 | OCH ₃ | CH ₂ OCOCH ₃ | -NH(CH ₂) ₃ COOt Bu | H | 0.1 |
| | " | " | -N(CH ₃)CH ₂ φ | H | 0.06 |
| | " | " | -Ot Bu | H | 0.5 |
| 20 | -OC ₆ H ₅ | " | " | H | 0.8 |
| | -F | " | " | H | 0.03 |
| | -Cl | " | " | H | 0.02 |

25

30

| | R ₁ | M | B | O | ED ₅₀ |
|----|--|--|--------------------------|---|------------------|
| | $\begin{array}{c} \text{O} \\ \\ -\text{OCH} \end{array}$ | " | " | H | 0.15 |
| 5 | $\begin{array}{c} \text{O} \\ \\ -\text{OCH} \end{array}$ | " | $-\text{OCH}_3$ | H | 0.1 |
| | $-\text{OCOCH}_3$ | " | $-\text{OCH}_2\emptyset$ | H | 0.4 |
| 10 | $-\text{OCH}_3$ | $-\text{CH}_2\text{OH}$ | $-\text{OtBu}$ | H | 0.8 |
| | " | $-\text{CH}_2\text{OCOCH}_2\text{CH}_2-\text{COOH}$ | " | H | 0.1 |
| 15 | " | $-\text{CH}_2\text{OCOCH}_2\text{NHCH}_3$ | " | H | 0.3 |
| | OCH_3 | $-\text{CH}_2\text{OCO}-\emptyset-(\text{O}-\text{COOH})$ | $-\text{OtBu}$ | H | 0.6 |
| 20 | " | $-\text{CH}_2\text{OCOCH}_2\text{NCOOtBu}$ $\quad\quad\quad $ $\quad\quad\quad \text{CH}_3$ | " | H | 0.1 |
| | " | $-\text{CH}_2\text{OCOOC}_2\text{H}_5$ | " | H | 0.3 |
| 25 | " | $-\text{CH}_2\text{Cl}$ | " | H | 0.15 |
| 30 | | | | | |

| | R ₁ | M | B | O | ED ₅₀ |
|----|-------------------|---|--------|---|------------------|
| 5 | " |  | " | H | 0.9 |
| | " |  | " | H | 0.7 |
| 10 | -OCH ₃ |  | -Ot Bu | H | 1.0 |
| | " | -CH ₂ OCH ₃ | " | H | 0.3 |
| 15 | " |  | " | H | 1.0 |
| | -OCH ₃ |  | -Ot Bu | H | 3.0 |
| 20 | " |  | " | H | 0.6 |
| 25 | " |  | " | H | 0.6 |
| 30 | | | | | |

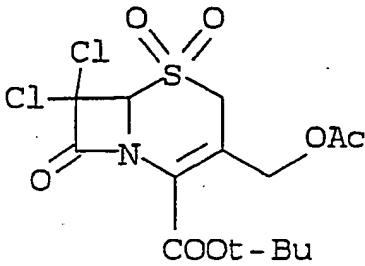
| | R ₁ | M | B | Q | ED ₅₀ |
|----|-------------------|--|--|---|------------------|
| | " | -CH ₂ OCONHCH ₂ COOH | " | H | 0.04 |
| 5 | " | -COOH | " | H | 0.01 |
| | " | -CH ₂ SØ | " | H | 0.4 |
| | " | -CH ₂ SOØ | " | H | 0.08 |
| 10 | " | -CH ₂ SO ₂ Ø | " | H | 0.2 |
| | -OCH ₃ | -Cl | -OCH ₃ | H | 0.2 |
| 15 | " | -CH ₂ SO ₂ CH ₃ | -OtBu | H | 0.1 |
| | -OCH ₃ | -CH ₃ | -OtBu | H | 1.0 |
| | " | -CH ₃ | -OCH ₂ COOC ₂ H ₅ | H | 0.5 |
| 20 | " | -CH ₃ | -OCH ₂ Ø-(mCOOCH ₃) | H | 0.3 |

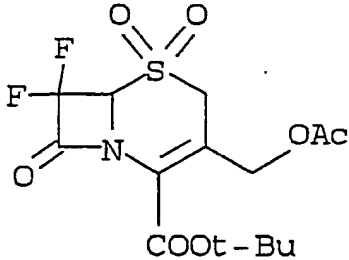
Ø = C₆H₅- or C₆H₄ i.e., phenyl

25

30

TABLE IIa

| 5 | <u>Compound</u> | <u>ED₅₀</u> |
|----|---|------------------------|
| 10 |  | 0.1 |

| | | |
|----|--|------|
| 15 |  | 0.05 |
|----|--|------|

20

25

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Protocol - Enzyme Assays for the Inhibition of Human Polymorphonuclear Leukocyte Elastase Via Hydrolysis of N-t-Boc-alanyl-alanyl-prolylalanine-p-nitroanilide

5 Reagents:

0.05M TES (N-tris[hydroxymethyl]methyl-2-amino-ethanesulfonic acid) Buffer, pH 7.5.

0.2 mM N-t-Boc-alanyl-alanyl-prolyl-alanine-p-nitroanilide (Boc-AAPAN).

10 To prepare substrate, the solid (m.w. 550) was first dissolved in 10.0 ml DMSO. Buffer at pH 7.5 was then added to a final volume of 100 ml.

Crude extract of human polymorphonuclear leukocytes (PMN) containing elastase activity.

15 Inhibitors (cephalosporin sulfone esters) to be tested dissolved in DMSO just before use.

Assay Procedure:

20 To 1.0 ml of 0.2 mM Boc-AAPAN in a cuvette, 0.01-0.1 ml of DMSO with or without inhibitor was added. After mixing, a measurement was taken at 410 mμ to detect any spontaneous hydrolysis due to presence of test compound. 0.05 Milliliters of PMN extract was then added and the δ OD/min at 410 mμ was measured and recorded. Beckman model 35 spectro-
25 photometer was used.

Results:

Results were reported as ED₅₀, i.e.,
effective dosage in micrograms per milliliter (µg/ml)
5 for 50% inhibition of the enzyme activity 2 minutes
after zero time.

Comments:

10 The elastase activity in the crude PMN
extract may vary from one preparation to another. A
control of each new batch is run, and the volume
added in the assay procedure is adjusted according to
activity.

15 Accordingly, compounds of formula I are
useful in the treatment of cancer including
including promyelocytic leukemia, acute myelogenous
leukemia (FAB M1 and FAB M2), acute promyelocytic
leukemia (FAB M3), acute myelomonocytic leukemia (FAB
20 M5), erythroleukemia, chronic myelogenous leukemia,
chronic myelomonocytic leukemia, chronic monocytic
leukemia and conditions associated with leukemia
involving activity of PMN neutral proteases e.g.
disseminated intravascular coagulation. Similarly,
25 compounds of formula I are useful for the inhibition
of protease 3, inhibition of elastase, inhibition of
proliferation of leukemia cells, promotion
differentiation of leukemia cells and remission of
the disease state of leukemia.

30 Moreover, as described above, such treatment
may optionally comprise the co-administration of an
agent including agents selected from the group
consisting of epsilon-aminocaproic acid, heparin,
trasyolol, prednisolone, cytosine arabinoside,
b-mercaptapurine, cytarabine, an anthracycline and a
vitamin A derivative.

For each of the uses, the compounds of Formula (I) and optional treatment agents, may be administered orally, topically, parenterally, by inhalation spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, dogs, cats, etc., the compounds of the invention are effective in the treatment of humans.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparation. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch,

or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc.

5 The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl
10 monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or
15 kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for
20 the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia;
25 dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long
30 chain aliphatic alcohols, for example heptadeca-ethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation

products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The said aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspension may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oils, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan mono-oleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent

or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

5 The compounds of Formula (I) or other treatment agent may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at 10 the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

15 For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the anti-inflammatory agents are employed.

20 The amount of each active ingredient (formula I or other treatment agent) that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 5 mg to 5 gm of active agent compounded with an appropriate and convenient amount of carrier material which may vary 25 from about 5 to about 95 percent of the total composition. For purposes of this specification, the range of 5 mg to 5 gm is specifically intended to include, but is not limited to the ranges of 5 mg to 2 gm; 25 mg to 2 gm; 25 mg to 1 gm; 25 mg to 500 mg; 30 100 mg to 500 mg. Dosage unit forms will generally contain between from about 25 mg to about 500 mg of active ingredient.

5 It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

10 The following example illustrates the preparation of the compounds useful in the method of treatment of the present invention, but does not limit the scope of the invention.

EXAMPLE 1

15 t-Butyl-3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of t-Butyl 3-acetyloxymethyl-7-diazo-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

20 Into a two-liter Erlenmeyer flask is placed a solution of 7-ACA ~~tert~~-butyl ester (7-ACA=3-acetyloxymethyl-7 β -amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (22.22 g; .067 mol;) in CH₂Cl₂ (500 ml). To this solution was added a
25 mixture of sodium nitrite (4.68 g, .067 mol) in water (500 ml). The resulting two-phase mixture was cooled in an ice bath, and then 2N aqueous H₂SO₄ (51 ml) was added dropwise over 30 minutes with vigorous
30 stirring. Stirring was continued for one hour at 0°, then the layers were separated and the aqueous layer was washed with methylene chloride (200 ml). The organic layers were combined, washed with brine (250 ml), dried over MgSO₄, and filtered to give a yellow solution of the diazo product which is used directly in the next reaction.

Step B: Preparation of t-Butyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

5 The solution of t-butyl 3-acetyloxymethyl-7-diazo-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate was cooled in an ice bath, and methanol (525 ml) was added. To this chilled mixture was added Rhodium (II) acetate dimer (210 mg), and the reaction mixture was stirred for 45 minutes, during 10 which time the color changes from yellow to green-brown. The reaction mixture was filtered through silica gel, concentrated and dried in vacuo to give a dark red oil which was then purified by preparative high-pressure liquid chromatography to 15 give 9.62 g (41.4%) of t-butyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate as a yellow oil.

20 Step C: Preparation of t-Butyl-3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide

25 In a 50 ml round bottom flask were placed t-butyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (2.07 g, 6.03 mmoles) and CH₂Cl₂ (25 ml). The resulting mixture was stirred under nitrogen with ice bath cooling, then meta-chloroperbenzoic acid (2.0 g, 80-90% pure) was added, the ice bath was removed, and 30 stirring was continued for two hours. The reaction mixture was diluted with ethyl acetate (50 ml),

5 filtered and then washed with saturated sodium
bicarbonate (100 ml), water (100 ml) and then
saturated brine (50 ml). The organic layer was dried
over MgSO_4 , and concentrated to give 2.20 g crude
product. This product was purified by preparative
HPLC using hexane:ethyl acetate (2:1) to give a white
solid further purified by recrystallization from
EtOAc/Hex to give 1.23 g (54.3%) of analytically pure
t-butyl-3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-
1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide
10 m.p. 127°.

Calcd. for $\text{C}_{15}\text{H}_{21}\text{NO}_8\text{S}$

| | C (%) | H | N | S |
|--------|-------|------|------|------|
| | 47.99 | 5.64 | 3.73 | 8.54 |
| Found: | 48.05 | 5.68 | 3.57 | 8.53 |

15 Following the same procedure described above
but substituting for the 7-ACA tert-butyl ester used
therein, t-butyl 3-methyl-7 β -amino-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate, there was
20 prepared 152 mg (24% yield) of t-butyl 3-methyl-
7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-
2-carboxylate.

25

30

EXAMPLE 2

t-Butyl-3-acetyloxymethyl-7 α -formyloxy-8-oxo-5-thia-
1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of t-Butyl 3-acetyloxymethyl-7 α -
hydroxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-
2-ene-2-carboxylate

5

Crude t-butyl 3-acetyloxymethyl-7-diazo-8-
oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
(prepared from 20.5 g, i.e. 63 mmole of t-butyl ester
of 7-ACA) was taken up in 400 ml of acetone and 400
ml of water containing 80 ml of 1N perchloric acid
was added at room temperature. The reaction was
stirred for 3 hours (or until nitrogen evolution
ceases) and was then diluted with water and extracted
twice with methylene chloride. The organic phases
were washed with saturated aqueous sodium bicarbonate
solution and saturated aqueous sodium chloride
solution, dried over sodium sulfate and evaporated in
vacuo. The residue was chromatographed on silica gel
with 30% ethyl acetate-hexane to give 2.75 g (13%) of
t-butyl 3-acetyloxymethyl-7 α -hydroxy-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate as a white
solid, NMR (CDCl₃) δ 1.53 (s, 9), 2.07 (s, 3), 3.35
(ABq, 2, 18 Hz), 4.5-5.0 (m, 4).

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20

25

Step B: Preparation of t-Butyl 3-acetyloxymethyl-7 α -
formyloxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct
-2-ene-2-carboxylate

30

To a solution of 1.5 g (4.6 mmols) of t-butyl
3-acetyloxymethyl-7 α -hydroxy-8-oxo-5-thia-1-aza-
bicyclo[4.2.0]oct-2-ene-2-carboxylate in 50 ml of
methylene chloride at 0°C was added 1.5 ml of

acetic-formic anhydride reagent (prepared by cooling 2 volumes of acetic anhydride to 0°C, slowly adding 1 volume of 96% formic acid, heating at 50° for 15 minutes and cooling) followed by 1.2 ml of pyridine. The reaction was allowed to warm to room temperature, stirred for 2 hours and then quenched by addition of ice water. The layers were separated and the organic layer was washed with saturated aqueous sodium bicarbonate solution and saturated aqueous sodium chloride solution, dried over sodium sulfate and evaporated in vacuo. The residue was triturated with 30% ethyl acetate-hexane to give 700 mg (43%) of t-butyl-3-acetyloxymethyl-7 α -formyloxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, NMR (CDCl₃) δ 1.53 (s, 9), 2.03 (s, 3), 3.40 (AB q, 2, 17 Hz), 4.67 (d, 1, 2 Hz) 4.78 (ABq, 2, 13 Hz), 5.49 (br d, 1, 2 Hz), 7.99 (s, 1).

Step C: Preparation of t-Butyl-3-acetyloxymethyl-7 α -formyloxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Following substantially the same procedure as described in Example 1, Step C, 750 mg of t-butyl 3-acetyloxymethyl-7 α -formyloxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate was oxidized to 545 mg (67%) of t-butyl-3-acetyloxymethyl-7 α -formyloxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide as a white solid, m.p. 171-172°C dec.

EXAMPLE 3

t-Butyl-3-acetyloxymethyl-7 α -acetyloxy-8-oxo-5-thia-
1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

5 Step A: Preparation of t-butyl 3-acetyloxymethyl-7 α -
acetyloxy-8-oxo-5-thia-1-azabicyclo[4.2.0]
oct-2-ene-2-carboxylate

To a solution of 240 mg (0.73 mmoles) of
t-butyl-3-acetyloxymethyl-7 α -hydroxy-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate in 10 ml of
10 methylene chloride at 0°C was added 85 mg (1.1 mmols)
of acetyl chloride and 90 mg (1.1 mmols) of
pyridine. The ice bath was removed and the reaction
was stirred at room temperature for 3 hours. The
reaction was then poured into ice water and the
layers separated. The organic layer was washed with
15 saturated aqueous sodium bicarbonate solution and
saturated aqueous sodium chloride, dried over sodium
sulfate, and evaporated. Chromatography on silica
gel with 30% ethyl acetate-hexane and trituration
with hexane gave 100 mg (37%) of t-butyl-3-
20 acetyloxymethyl-7 α -acetyloxy-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate as a white
solid, m.p. 94-95°C with decomposition.

Step B: Preparation of t-Butyl-3-acetyloxymethyl-
7 α -acetyloxy-8-oxo-5-thia-1-azabicyclo
25 [4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Following the same procedure as described in
Step C, Example 1, 100 mg of t-butyl-3-acetyloxy-
methyl-7 α -acetyloxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-
oct-2-ene-2-carboxylate was oxidized to t-butyl-3-
30 acetyloxymethyl-7 α -acetyloxy-8-oxo-5-thia-1-abi-
cyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide m.p.
126-129°C.

EXAMPLE 4

Methyl-3-chloro-7 α -methoxy-8-oxo-5-thia-1-azabicyclo
[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of Methyl 3-ethoxycarbonylthia-
methyl-7 β -amino-8-oxo-5-thia-1-azabicyclo
5 [4.2.0]oct-2-ene-2-carboxylate

To a vigorously stirred suspension of 8 g
(23.9 mmols) of 3-ethoxycarbonyl-thiamethyl-7 β -amino-
8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic
acid in 300 ml of methanol was slowly added a
10 solution of diazomethane in ethyl ether until most of
the solid dissolves. The excess diazomethane was
quenched after 5 minutes by addition of acetic acid.
The reaction was then poured into ice water and
extracted twice with ethyl acetate/ethyl ether
15 (1:1). The organic layers were washed with water and
saturated aqueous sodium chloride solution, dried
over sodium sulfate and evaporated in vacuo.

The residue was flash chromatographed
eluting with a solvent gradient of 50 to 70% ethyl
20 acetate-hexane to give 4.5 g (54%) of methyl 3-
ethoxycarbonylthiamethyl-7 β -amino-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate as a white
solid, NMR (CDCl₃) δ 1.40 (t, 3, 7 Hz), 1.73 (br s,
1) 3.53 (ABq, Z, 19 Hz), 3.87 (s, 3), 4.30 (ABq, 2,
25 13 Hz), 4.5-5.0 (m, 4).

Step B: Preparation of Methyl 3-ethoxycarbonyl-
thiamethyl-7 α -methoxy-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate

Following substantially the same procedures
30 as described in Example 1, Steps A and B, 4.5 g (12.9
mmoles) of methyl 3-ethoxycarbonylthiamethyl-7 β -
amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-

carboxylate were converted to 1.2 g (26%) of methyl 3-ethoxycarbonylthiamethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate as a yellowish solid, NMR (CDCl₃) δ 1.38 (t, 3, 7 Hz), 3.43 (ABq, 2, 18 Hz), 3.51 (s, 3), 3.86 (s, 3H), 4.22 (HBq, 2, 13 Hz), 4.3-4.7 (m, 4).

Step C: Preparation of Methyl 3-methylene-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

To a suspension of 8 g of Raney nickel in 70 ml of water under nitrogen was added 1.2 g (3.3 mmoles) of methyl 3-ethoxycarbonylthiamethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate in 70 ml of ethanol.

The mixture was hydrogenated at 40 psi of hydrogen in a Parr shaker at room temperature for 16 hours. The catalyst was then removed by filtration and the filtrate was diluted with water and extracted twice with ethyl acetate. The organic layers were washed with water and saturated aqueous sodium chloride solution, dried over sodium sulfate and evaporated in vacuo. The residue was flash chromatographed eluting with a solvent gradient of 30 to 40% ethyl acetate-hexane to give 500 mg (62%) of methyl 3-methylene-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate as a colorless oil, NMR (CDCl₃) δ 3.33 (ABq, 2, 14 Hz), 3.56 (s, 3), 3.73 (s, 3), 4.40 (br s, 1), 5.0-5.3 (m, 4).

Step D: Preparation of Methyl 3-hydroxy-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

A solution of 200 mg (0.82 mmols) of methyl 3-methylene-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate in 15 ml of methylene chloride was cooled to -70°C in a dry ice-acetone bath. Ozone was bubbled through the solution until the first sign of blue coloration was noticed. Nitrogen was then bubbled through to flush out the excess ozone, 2 g of sodium bisulfite were added and the suspension was vigorously stirred at 0°C for 30 minutes.

The reaction was filtered and the filtrate was evaporated in vacuo to give crude product which was used directly in the next step.

Step E: Preparation of Methyl 3-chloro-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

The crude methyl 3-hydroxy-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (approximately 0.82 mmols) was taken up in 10 ml of dimethylformamide and slowly added to a solution of 680 mg (3.3 mmols) of phosphorous pentachloride in 10 ml of dimethylformamide at -60°C (prepared by stirring at -10°C for 15 minutes, then cooling to -60°C). The solution was stirred at -60°C for 30 minutes, then at -10°C for 90 minutes before it was quenched by pouring into water/ethyl acetate. The organic layer was washed with saturated aqueous sodium bicarbonate solution and saturated aqueous sodium chloride solution, dried over sodium sulfate and evaporated in vacuo to give a crude residue of methyl 3-chloro-7 α -methoxy-8-oxo-5-thia-1-

azabicyclo[4.2.0]oct-2-ene-2-carboxylate. This was oxidized directly to afford 4.9 mg of methyl 3-chloro-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide (according to procedures described in Example 1, Step C.) NMR (CDCl₃) δ 3.57 (s, 3), 3.90 (s, 3), 4.00 (ABq, 2, 18 Hz), 4.67 (brs, 1), 5.10 (d, 1, 2 Hz).

EXAMPLE 5

Methyl 3-phenylthio (or 3-phenylsulfonyl)-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of Methyl 3-tosyloxy-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

A solution of 450 mg of crude methyl 3-hydroxy-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate in 5 ml of pyridine was stirred with 470 mg (2.4 mmols) of tosyl chloride at 0°C for 3 hours. The reaction mixture was poured into ice water and extracted twice with ethyl acetate. The organic layers were washed twice with dilute aqueous hydrochloric acid and saturated aqueous sodium chloride, dried over sodium sulfate and evaporated in vacuo to give a crude residue of methyl 3-tosyloxy-7 α -methoxy-8-oxo-5-thio-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. This is directly oxidized in accordance with the same procedure of Example 1, Step C to give 180 mg (20%) of methyl 3-tosyloxy-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide as a white solid, NMR (CDCl₃) δ 2.47 (s, 3), 3.57 (s, 3), 3.70 (s, 3), 4.10 (ABq, 2, 18 Hz), 4.67 (m, 1), 5.07 (d, 1, 2 Hz), 7.50 (ABq, 4, 9 Hz).

Step B: Preparation of Methyl 3-phenylthio-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

A solution of 170 mg (0.40 mmols) of methyl 3-tosyloxy-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide in 2 ml of N,N-dimethylformamide was stirred with 50 μ l (0.40 mmols), of thiophenol and 50 μ l (0.40 mmols) of N-ethyl-N,N-diisopropylamine at -10°C for 30 minutes. The reaction was poured into ice water and extracted twice with ethyl acetate. The organic layers were washed with dilute hydrochloric acid and saturated aqueous sodium chloride solution, dried over sodium sulfate and evaporated. The residue was chromatographed on 2 x 2000 μ m silica preparative plates using 40% ethyl acetate-hexane. Since the product band still contained some of the higher R_f impurity (the major product), it was rechromatographed on a 1000 μ m silica preparative plate to give 15 mg (10%) of methyl 3-phenylthio-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide, NMR (CDCl₃) δ 3.53 (s, 3), 3.57 (ABq, 2, 18 Hz), 3.87 (s, 3), 4.51 (br s, 1), 5.00 (d, 1, 2 Hz), 7.33 (br s, 5).

Step C: Preparation of Methyl 3-phenylsulfonyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

A solution of 11 mg (0.030 mmole) methyl 3-phenylthio-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide in 1 ml of methylene chloride was stirred with 12 mg (0.060 mmols) of m-chloroperbenzoic acid at 0°C for 30 minutes. The entire reaction was then chromatographed

on a 1000 μ m silica preparative plate eluting with 50% ethyl acetate-hexane to give 8 mg (67%) of methyl 3-phenylsulfonyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide. NMR (CDCl₃) δ 3.36 (d, 1, 18 Hz), 3.53 (s, 3), 3.96 (s, 3), 4.20 (br d, 1, 18 Hz), 4.70 (br s, 1), 5.06 (d, 1, 2 Hz), 7.2-7.8 (m, 5).

EXAMPLE 6

t-Butyl 3-acetyloxymethyl-7 α -fluoro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of t-Butyl 3-acetyloxymethyl-7 α -fluoro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

t-Butyl 3-acetyloxymethyl-7-diazo-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate was prepared from 10 mmoles 7-amino derivatives in the same manner as described in Step A, Example 1, and taken up in 25 ml dry methylene chloride. To it with stirring was added dropwise over 30 seconds 0.60 ml 70% HF in pyridine. The mixture was stirred 2.5 minutes more and then washed with aq K₂HPO₄, water, aq H₃PO₄ and brine. It was dried with MgSO₄, filtered and chromatographed on 164 silica gel with 1:1 hexane-ethyl acetate, affording 183 mg of t-butyl 3-acetyloxymethyl-7 α -fluoro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. IR(μ): 5.57, 5.76. NMR (CDCl₃): δ 1.54 s, t-Bu; 2.08 s, Ac; 3.34 d of d, J = 18, 1.9 Hz, and 3.58 d of d, J = 18, 0.8 Hz, SCH₂; 4.75 d, J = 13 Hz and 4.97 d, J = 13 Hz, CH₂OAc; 4.90 d of d, J = 9, 1.6 Hz, CHS; 5.32 d of d, J = 54, 1.6 Hz, CHF. MS: 332.

Step B: Preparation of t-Butyl 3-acetyloxymethyl-7 α -fluoro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

To 182 mg (0.55 mmol) t-Butyl 3-acetyloxymethyl-7 α -fluoro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate in 20 ml methylene chloride was added 261 mg (1.2 mmol) MCPBA. After stirring 5.5 hours, the mixture was washed with aq K₂HPO₄ and brine, dried with MgSO₄, filtered, evaporated and chromatographed by PLC on silica gel with 1:1 hexane-EtOAc, affording 142 mg t-Butyl 3-acetyloxymethyl-7 α -fluoro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide. NMR (CDCl₃): δ 1.55 s, t-bu; 2.08 s, Ac; 3.73 d, 3.90 d, J = 18 Hz, SO₂CH₂; 4.72 d, 4.90 d, J = 13 Hz, CH₂OAc; 4.86 d of d, J = 7, 1.6 Hz, CHSO₂; 5.84 d of d, J = 52, 1.6 Hz, CHF. MS: 307, 247.

EXAMPLE 7

t-Butyl 3-acetyloxymethyl-7 α -chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of t-Butyl 3-acetyloxymethyl-7 α -chloro-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

Following the same procedure as described in Step A, Example 1, 7-AcA t-butyl ester was diazotized to t-Butyl 3-acetyloxymethyl-7-diazo-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate which was taken up into 2 ml EtOH, and treated with 0.1 ml aq HCl. There was an instantaneous vigorous effervescence. After 15 seconds, aq K₂HPO₄ and methylene chloride were added. The methylene chloride layer was separated, washed with aq

H₃PO₄ and brine, dried with MgSO₄, filtered and chromatographed by PLC on silica gel, eluting with 25:1 CHCl₃-EtOAc, to provide 61 mg pure t-Butyl 3-acetyloxymethyl-7 α -chloro-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. NMR (CDCl₃): δ 1.55 s, t-bu; 2.10 s, Ac; 3.40 d, 3.59 d, J = 18 Hz, SCH₂; 4.79 d, 5.03 d, J = 13 Hz, CH₂OAc; 4.70 d, J = 1.5 Hz, CHS; 4.78 d, J = 1.5 Hz, CHCl. MS: 291 Cl₁, 231 Cl₁.

Step B: Preparation of t-Butyl 3-acetyloxymethyl-7 α -chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

4.5 mg (0.013 mmol) t-Butyl 3-acetyloxymethyl-7 α -chloro-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate was stirred with 6.0 mg MCPBA (0.028 mmol) in 0.5 ml methylene chloride for 4 hours. After the first 2 hours, and again after 3.5 hours, 1 mg additional MCPBA was added. The mixture was washed with aq K₂HPO₄, dried with MgSO₄, filtered and chromatographed on 500 mg silica gel, eluting with 1:1 hexane-EtOAc, 3.2 mg of t-Butyl 3-acetyloxymethyl-7 α -chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide. MS: 379 Cl₁. NMR (CDCl₃): δ 1.57 s, t-bu; 2.08 s, Ac; 3.83 d, 4.00 d, J = 18, SO₂CH₂; 4.69 d, 5.00 d, J = 13 Hz, CH₂OAc; 4.80 d, J = 2 Hz, CHSO₂; 5.26 d, J = 2 Hz, CHCl.

EXAMPLE 8

t-Butyl 3-hydroxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate

Step A: Preparation of t-Butyl 3-acetyloxymethyl-
7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]
oct-3-ene-2-carboxylate

In a 200 ml round bottom flask equipped with
a magnetic stirrer were placed t-Butyl 3-acetyloxy-
methyl-7 α -methoxy-8-oxo-5-thio-1-azabicyclo[4.2.0]
oct-2-ene-2-carboxylate (7.46 g, 21.7 mmol)
chloroform (90 ml) and triethylamine (3.3 ml, 23.6
mmol). The mixture was then heated to reflux for
three hours. ^1H NMR analysis of an aliquot shows
that there was a mixture of isomers (-3-ene
and-2-ene) in the approximate ratio of 3:1. The
reaction mixture was then evaporated and the brown
residue was dried in vacuo. The mixture was then
purified by preparative HPLC on a Waters Prep 500
using two silica gel columns in series and
hexane:ethyl acetate (3:1) as eluent. The forecut of
the partially resolved material was taken and
evaporated to give 2.41 g (32%) of pure t-Butyl
3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-3-ene-2-carboxylate isomer as a
yellow oil. ^1H NMR (CDCl_3): δ 6.45 (4-H, br, s),
5.05 (2-H, br s), 4.90 (7-H, d, $J = 2$), 4.65 (6-H, d,
 $J = 2$), 4.60 (CCH_2OAc , t, $J = 13$ Hz), 3.52 (OCH_3 , s),
203 (O_2CCH_3 , s), 1.50 (s, $\text{OC}(\text{CH}_3)_3$).

Collection of the remainder of the material
and evaporation yielded 3.66 g (49%) of a 1:1 mixture
of 2-ene and 3-ene isomers as a yellow oil which may
be reused in the reaction.

Step B: Preparation of t-Butyl 3-hydroxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate

To a solution of 8.88 g (25.9 mmol) of t-Butyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate in isopropanol (100 ml) was added Ti(O-*i*pr)₄ (5.4 ml). The reaction mixture was heated to reflux under N₂ and monitored by TLC [silica gel using hexane:ethyl acetate (1:1); starting material R_f 0.75, product R_f 0.5] until the starting material had just disappeared. The reaction was concentrated and the residue was dissolved in ethyl acetate and washed with 1N aqueous H₃PO₄ (50 ml). The aqueous layer was then backwashed with ethyl acetate (50 ml) and the organic layers were combined, washed with water (50 ml) and brine (50 ml). The organic layer was dried over MgSO₄ and concentrated to give a yellow oil which was purified using a Waters Prep 500 with hexane:ethyl acetate (2:1) as eluent to give 4.76 g (61%) of a light yellow oil which on standing crystallizes to t-Butyl 3-hydroxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate. ¹H NMR (CDCl₃): δ 6.32 (4-H, m); 5.02 (2-H, s); 4.99 (6-H, J = 1 Hz), 4.60 (7-H, d J = 1 Hz); 4.22 (CCH₂OH, br s); 3.53 (OCH₃, s); 2.60 (OH, br exch.); 1.50 (-CO₂C-(CH₃)₃, s).

EXAMPLE 9

t-Butyl 3-(3-[hydroxycarbonyl]propanoyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of t-Butyl 3-(3-[hydroxycarbonyl]propanoyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate

A mixture of t-Butyl 3-hydroxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-3-ene-2-carboxylate (602 mg, 2.0 mmol) and succinic anhydride (300 mg, 3.0 mmol) were dissolved in dry tetrahydrofuran (4 ml) under nitrogen at room temperature, then 4-[N,N-dimethylamino]pyridine (300 mg, 2.5 mmol) was added with stirring. A solid began to separate out shortly after mixing. The mixture was allowed to stir 15 hours, then 50% saturated aqueous sodium bicarbonate (10 ml) was added, and the mixture was extracted with ether (2 x 20 ml). The combined ether extracts were washed with 50% sat. aq NaHCO₃ (10 ml), then the aqueous extracts were combined and acidified to pH 2.5 (using 1.0 M.H₃PO₄), the resulting cloudy solution was extracted with ethyl acetate (2 x 30 ml), then the organic layers were combined and washed with saturated brine (25 ml) and dried over Na₂SO₄. The solvent was removed in vacuo to give t-Butyl 3-hydroxycarbonylethyl-carbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate a yellow oil. This material was sufficiently pure to be carried on to the next step.

Step B: Preparation of t-Butyl 3-(3-[hydroxycarbonyl]propanoyloxymethyl)-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

5 The crude product t-Butyl 3-hydroxycarbonyl-ethylcarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate from the above reaction was dissolved in methylene chloride (10 ml) and cooled to 0° under N₂. Then meta-
10 chloroperbenzoic acid (1.0 g, 5 mmol assuming 85% purity) was added all at once, and after stirring for 5 minutes, the cooling bath was removed. Stirring was continued for five hours, then the reaction mixture was filtered and the filter cake was washed with ice-cold CH₂Cl₂ (5 ml). The combined filtrates
15 were evaporated and the residue was dissolved in CH₂Cl₂/EtOAc (3:1) (8 ml) and chromatographed on silica gel (4 X [20 cm x 20 cm] 2000 μ silica gel GF using 1% HOAc in 1/1 EtOAc/Hex as eluent) the bands at R_f 0.3 were removed, combined and eluted with 1%
20 HOAc in EtOAc, and the eluent was evaporated to give a clear oil. This material was lyophilized from benzene to remove HOAc, then crystallized from ether/hexane to yield 502 mg of product t-Butyl
25 3-(3-[hydroxycarbonyl]propanoyl-oxymethyl)-7 α -methoxy-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide (58%) m.p. 112-113°.

EXAMPLE 10

t-Butyl 3-hydroxycarbonylmethylaminocarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

5 Step A: Preparation of t-Butyl-3-(p-methoxybenzyloxy) carbonylmethylaminocarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate

10 To a solution of t-Butyl 3-hydroxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-3-ene-2-carboxylate (602 mg, 2.0 mmol) in methylene chloride (5 ml) was added N,N'-carbonyl diimidazole (324 mg, 2.0 mmol). The resulting solution was stirred at room temperature for thirty minutes, then the solvent was removed in vacuo and the residue was
15 dissolved in N,N-dimethylformamide (5 ml). Then glycine p-methoxybenzylester hydrochloride (693 mg, 3.0 mmol) and 4-(N,N-dimethylamino)pyridine (366 mg, 3.0 mmol) were added, and the resulting mixture was stirred for 18 hours. The reaction mixture was then
20 poured into water (30 ml) and extracted with ethyl acetate (2 x 30 ml). The combined organic layers were washed with water (20 ml) and saturated brine (20 ml), then dried over sodium sulfate, and the solvent was removed to give a residue which was
25 purified by chromatography (4x[20 cm x 20 cm] 2000 μ silica gel GF plates, developed in ethyl acetate/hexane [1/1]. The bands at Rf 0.55 were removed and eluted with ethyl acetate to produce 508
30 mg (51%) of t-Butyl-3-(p-methoxybenzyloxy)carbonylmethylaminocarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate. ¹H NMR

(CDCl₃) δ 7.25-6.85 (C₆H₄OCH₃, d, d, J = 9Hz); 6.40 (C=C(H)S-, br. s); 5.40 (=NH, br, s); 5.18 (-C₆H₄-CH₂-, s); 5.05 (-CH-CH=CH), s); 4.90 (C-6H, d, J = 2Hz); 4.70 (C-7H, d, J = Hz); 4.65 (C-3-CH₂, t, J = 12Hz); 3.95 (-HN-CH₂CO-, d, J = 6 Hz); 3.80 (-C₆H₄-OCH₃, s); 3.55 (C-7-OCH₃, s); 1.55 [-COO-C(CH₃)₃].

Step B: Preparation of t-Butyl 3-(p-methoxybenzyloxy) carbonylmethylaminocarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Following substantially the same procedures as described in Example 9, Step B, 278 mg (0.56 mmole) of t-Butyl-3-(p-methoxybenzyloxy)carbonylmethylaminocarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate was oxidized to t-Butyl-3-(p-methoxybenzyloxy)carbonylmethylaminocarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide. (226 mg, 76%) as a glass. ¹H NMR δ (CDCl₃) 7.28-6.85 -C₆H₄OCH₃, d, d, J = 9); 5.55 (CONH-, br t, J = 6); 5.19 (C-6 or 7H, d, J = 2); 5.10 (-C₆H₈-CH₂O-, s); 4.85 (C-3-CH₂O-), d of cl, J = 12, 26); 3.90 (C₂-CH₂, -4N-CH₂COO-, m); 3.79 (-C₆H₄-OCH₃, s); 3.55 (C-7(OCH₃), s); 1.55 (-CO₂C(CH₃)₃, s).

Step C: Preparation of t-Butyl 3-hydroxycarbonylmethylaminocarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

A mixture of trifluoroacetic acid and anisole (5:1) was cooled to 0°, then one milliliter of this solution was added to a flask under N₂ at 0° containing t-butyl 3-(p-methoxybenzyloxy)carbonyl-

methylaminocarbonyloxymethyl-7 α -methoxy-8-oxo-5-thia-
1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide
(105 mg, 0.2 mmol). The mixture was stirred

vigorously with cooling until it becomes

homogeneous. Stirring was continued for 2 minutes,
then the flask was attached through a gas inlet tube
to a vacuum pump in order to remove solvent as fast
as possible with continued cooling. Cooling was

maintained until the reaction mixture becomes

noticeably viscous. The cooling bath was removed and
pumping was continued for one hour, then the residue

was dissolved in a methylene chloride (one ml) and
chromatographed (20 x 20 cm 2000 μ silica gel GF

plate using 1% HOAc in ethylacetate/hexane (1/1) as
eluent). The band at Rf 0.25 was removed and eluted

with 1% HOAc in ethyl acetate. The solvent was
removed and the residue was lyophilized from benzene

to remove residual HOAc to obtain t-Butyl 3-hydroxy-
carbonylmethylaminocarbonyloxymethyl-7 α -methoxy-8-
oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-
5,5-dioxide as an amorphous solid (36 mg, 44% yield)

^1H NMR (CDCl_3); δ 6.95 (CO_2H , br exch.); 5.60
($-\text{CONHCH}_2-$, br exch.); 5.20 (C_6 or C_7 H, J = 2);

O H

5.90 ($\text{C}_3(\text{CH}_2\text{O}-\text{C}-\text{N}-)$ br d of d, J = 12, 22) 5.78 (C_6
or C_7 H, br s) 3.90 (C_4-H and HNCH_2COOH , m); 3.55
($\text{C}_7(\text{OCH}_3)$, s); 1.55 ($\text{CO}_2\text{C}(\text{CH}_3)_3$, s).

EXAMPLE 11

t-Butyl 3-hydroxycarbonyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of t-Butyl 3-formyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

To a 100 ml recovery flask under nitrogen was added solution of t-Butyl 3-hydroxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (300 mg 1.0 mmol) in acetone (26 ml-freshly distilled from Jones' Reagent). The solution was then cooled to 0°, and Jones Reagent (1.0 mmol, 372 μ l of a 2.7 M solution) was added slowly with stirring. There was an immediate color change from yellow to green with precipitate formation. After the addition was complete, stirring was continued for ten minutes, then it was poured into water (100 ml) and stirred. The resulting green solution was extracted with ethyl acetate (4 x 30 ml), and the combined organic extracts were washed with brine, then dried with sodium sulfate. The solvent was removed to give a yellow oil (264 mg) which was purified by chromatography (2000 μ silica gel GF prep plate [20 x 20 cm] using ethyl acetate:hexane (1:1) as eluent). The band of Rf 0.5 was removed to give 214 mg (72%) of t-Butyl 3-formyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate as a clear oil which solidified on cooling. ^1H NMR (CDCl_3): δ 9.40 (-CHO, s); 7.55 ($\text{C}_4\text{-H}$, d, J = 1 Hz); 5.30 (C_6 or $\text{C}_7\text{-H}$, d, J = 1); 5.08 ($\text{C}_2\text{-H}$, d, J = 1); 4.65 (C_6 or $\text{C}_7\text{-H}$, d, J = 1); 3.55 ($\text{C}_7(\text{OCH}_3)$, s); 1.55 ($\text{CO}_2\text{C}(\text{CH}_3)_3$ m s).

Step B: Preparation of t-Butyl 3-hydroxycarbonyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate-5,5-dioxide

Into a 100 ml recovery flask under nitrogen was placed a solution of t-Butyl 3-formyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (425 mg, 1.42 mmol) in acetone distilled from Jones' Reagent (35 ml). To this solution was added Jones' Reagent (1.6 ml of a 2.7 M solution, 4.2 mmol) dropwise over 15 minutes. Stirring was continued for three hours, then the reaction was diluted with water (100 ml) and the resulting green solution was extracted with ethyl acetate (2 x 75 ml). The combined organic extracts were washed with saturated brine (50 ml) and dried over magnesium sulfate. The solvent was removed in vacuo to give a yellow oil which was purified by silica gel prep. plate chromatography (2 x 2000 μ using 1% HOAc in ethyl acetate/hexane [1/1]). The band at RF 0.3 is removed and eluted to give 196 mg (44%) of t-butyl 3-hydroxycarbonyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate as a clear oil. ¹H NMR (CDCl₃); 9.90 (CO₂H, br, s, exch.); 7.72 (C₄-H, d, J = 1.5 Hz); 5.3 (C₆ or C₇-H, d, J = 1), 4.95 (C₂-H, br, s); 4.65 (C₆ or C₇-H, d, J = 1); 3.55 (C₆(OCH₃), s); 1.50 (CO₂C(CH₃)₃, s).

Following substantially the same procedure as described in Example 9, Step B, t-Butyl 3-hydroxycarbonyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (196 mg, 0.62 mmol) was oxidized to afford 96 mg (46%) of t-Butyl 3-hydroxycarbonyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]

oct-2-ene-2-carboxylate-5,5-dioxide as a white solid. ^1H NMR (CDCl_3); δ 8.65 (CO_2H , br, s); 5.30 (C_6 or $\text{C}_7\text{-H}$, d, $J = 3$ Hz); 5.05 (C_6 or $\text{C}_7\text{-H}$, br, d, $J = 3$); 4.05 ($\text{C}_4\text{-H}_2$, br, s); 3.55 ($\text{C}_7(\text{OCH}_3)$, s); 1.58 ($\text{CO}_2\text{C}(\text{CH}_3)_3$, s).

EXAMPLE 12

t-Butyl 3- chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of t-Butyl 3-chloro-methyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate

To a solution of 0.9 g (3 mmol) of t-Butyl 3-hydroxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate in 20 ml of tetrahydrofuran was added 1 ml of pyridine. Thionyl chloride (0.5 ml) was added dropwise over 5 min. After stirring the reaction mixture for 0.5 hours, it was poured into ice-cold water and extracted with ethyl acetate. The combined extract was washed with 7% sodium bicarbonate solution, brine and dried over sodium sulfate. The concentrated filtrate was flash chromatographed with 10% ethyl acetate-hexane to yield 0.626 g (65%) yield of t-butyl 3-chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate as a pale yellow solid. m.p. 85°.

Step B: Preparation of t-Butyl 3-chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Following substantially the same procedures as described in Example 9, Step B, 0.32 g (1 mmol) of

t-Butyl 3- chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate was oxidized to afford 0.301 g (86% yield) of t-Butyl 3- chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide, m.p. 132°C.

EXAMPLE 13

t-Butyl 3-phenylthio (or 3-phenylsulfonyl)methyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Step A: Preparation of t-Butyl 3-phenylthiomethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

A solution of 35 mg (0.24 mmol) of potassium thiophenoxide in 0.5 ml of water was added to a solution of 83 mg (0.24 mmol) of t-Butyl 3- chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide in 2 ml of acetone. After stirring the reaction mixture for 40 hours it was concentrated in vacuo. The residue was dissolved in ethyl acetate and poured into 7% sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine and dried over sodium sulfate. The concentrated filtrate was chromatographed on a preparative silica gel plate using 20% ethyl acetate-hexane to obtain 66 mg (66% yield) of t-butyl 3-phenylthiomethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide NMR(CDCl₃): δ 1.45 (s, 9), 3.46 (s, 3), 3.58 (d, 1, 14 Hz), 3.62 (d, 1, 18 Hz), 4.08 (d, 1, 18 Hz), 4.18 (d, 1, 14 Hz), 4.4 (bs, 1), 5.02 (d, 1, 2 Hz), 7.1-7.4 (m, 5).

(continued)

Step B: Preparation of t-Butyl 3-phenylsulfinylmethyl
or 3-phenylsulfonylmethyl-7 α -methoxy-8-oxo-
5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-
carboxylate-5,5-dioxide

5 A solution of 40 mg (90%, 0.21 mmol) of
m-chloroperbenzoic acid in 1 ml of dichloromethane
was added to a solution of 60 mg (0.14 mmol) of
t-Butyl 3-phenylthiomethyl-7 α -methoxy-8-oxo-5-thia-
1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide
10 while cooling in an ice bath. The reaction mixture
was stirred for 0.5 hour as it warmed to room
temperature. The solution was poured into 7% sodium
bicarbonate solution containing excess sodium sulfite
and extracted with dichloromethane. The combined
15 dichloromethane layers was washed with brine and
dried over sodium sulfate. The concentrated filtrate
was chromatographed on a preparative silica gel plate
with 50% ethyl acetate-hexane to give two bands.
The less polar band yielded 32 mg (50% yield) of
20 t-Butyl 3-phenylsulfonylmethyl-7 α -methoxy-8-oxo-5-
thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-
dioxide. NMR(CDCl₃): δ 1.46 (s, 9), 3.52 (s, 3),
3.81 (d, 2, 14 Hz), 3.82 (d, 2, 18 Hz), 4.32 (d, 1,
18Hz), 4.74 (d, 1, 14 Hz), 4.76 (bs, 1), 5.09 (d,
25 1, 2 Hz), 7.2-7.9 (m, 5). The more polar band
yielded 27 mg (44% yield) of t-Butyl
3-phenylsulfinylmethyl-7 α -methoxy-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide.
NMR(CDCl₃): δ 1.51 (s, 9), 3.53 (s, 3), 3.3-4.1 (m,
30 4), 4.53 (bs, 0.5), 4.67 (bs, 0.5), 5.07 (d, 1, 2
Hz), 7.3-7.7 (m, 5).

EXAMPLE 14

t-Butyl 3-methoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

5 A solution of 30 mg (0.09 mmol) of t-butyl 3-chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate in 1 ml of methanol was stirred at room temperature for 16 hours. The solution was concentrated in vacuo and the residue was chromatographed on a short silica gel column with 20% ethyl acetate-hexane to obtain 21 mg t-butyl 3-methoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabi-
10 cyclo[4.2.0]oct-3-ene-2-carboxylate. NMR (CDCl₃): δ 3.25 (s, 1.5) 3.39 (s, 1.5), 6.23 (bs, 0.5).

Following substantially the same procedure as described in Example 9, Step B, 21 mg of t-butyl 3-methoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo-
15 [4.2.0]oct-3-ene-2-carboxylate was oxidized to obtain 11 mg (34% yield) of t-butyl 3-methoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide. NMR (CDCl₃): δ 1.53 (s, 9), 3.27 (s, 3), 3.53 (s, 3), 3.72 (ABq, 2, 17 Hz),
20 4.14 (s, 2), 4.6 (bs, 1), 5.05 (d, 1, 2 Hz).

25

30

EXAMPLE 15

t-Butyl 3-methyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo
[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

5 To a solution of 5 g (14.0 mmol) of t-butyl-
3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo
[4.2.0]oct-2-ene-2-carboxylate in 100 ml of ethanol
was added 15 g of 10% palladium on carbon under a
nitrogen atmosphere. The solution was hydrogenated
on a Parr apparatus for 3 hr. The reaction mix was
10 filtered and the catalyst was thoroughly washed with
warm methanol. The filtrate and washings were
combined and concentrated in vacuo. The residue was
flash-chromatographed using 20% ethylacetate-hexane
to obtain 1.97 g (47% yield) of t-butyl 3-methyl-7 α -
methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-
15 carboxylate. NMR (CDCl₃): δ 1.53 (s, 9H), 2.01 (s,
3), 3.27 (ABq, 2, 18 Hz), 3.52 (s, 3), 4.4 (d, 1, 2
Hz), 4.6 (d, 1, 2 Hz).

Following substantially the same procedure
as described in Example 1, Step C 11 mg (0.04 mmol)
20 of t-butyl-3-methyl-7 α -methoxy-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate was oxidized
to obtain 6 mg (48% yield) of t-Butyl 3-methyl-7 α -
methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-
25 carboxylate-5,5-dioxide. NMR (CDCl₃): δ 1.53 (s,
9), 2.0 (s, 3), 3.53 (s, 3), 3.6 (ABq, 2, 12 Hz),
4.54 (bs, 1), 5.05 (bs, 1).

EXAMPLE 16

m-Methoxycarbonylbenzyl-3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

5 Step A: Preparation of N,N'-diisopropyl-O-(m-methoxycarbonylbenzyl)-isourea

A mixture of 4.75 g (28.6 mmols) of m-methoxycarbonylbenzyl alcohol and 3.6 g (28.6 mmols) of N,N'-diisopropylcarbodiimide was stirred with 50 mg (0.51 mmols) of cuprous chloride at room temperature for 24 hours. The reaction was then diluted with 10 ml of hexane and eluted through a short column of neutral alumina with 20% ethyl acetate-hexane to give 8.0 g (96%) of N,N'-diisopropyl-O-(m-methoxycarbonylbenzyl)-isourea as a colorless oil.

Step B: Preparation of m-methoxycarbonyl-benzyl-3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

20 A solution of 1.0 g (3.4 mmole) of N,N'-diisopropyl-O-(m-methoxycarbonylbenzyl)-isourea and 1.0 g (3.4 mmole) of 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid in 2.0 ml of tetrahydrofuran (THF) was stirred for 24 hours at room temperature. The reaction was then cooled to -10°C, filtered and concentrated in vacuo. The product was purified by flash chromatography using a solvent gradient of 35 to 40% ethyl acetate-hexane to give 300 mg (20%) of m-methoxycarbonylbenzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate as an oil, NMR (CDCl₃); δ 2.04 (s, 3), 3.40 (ABq, 2, 18 Hz) 3.50 (s, 3), 3.87 (s, 3), 4.47 (d, 1,

2 Hz), 4.63 (d, 2, 2 Hz), 4.78 (ABq, 2, 13 Hz), 5.27 (ABq, 2, 13 Hz), 7.2-7.6 (m, 2), 7.7-8.0 (m, 2).

Step C: Preparation of m-methoxycarbonylbenzyl-3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

Following the same procedure as described in Example 1, Step C, 250 mg (0.57 mmole) of m-methoxycarbonylbenzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate was oxidized to 210 mg (78%) of M-methoxycarbonylbenzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide, NMR (CDCl₃); δ 2.03 (s, 3), 3.52 (s, 3), 3.87 (ABq, 2, 19 Hz), 3.90 (s, 3), 4.67 (d, 1, 2 Hz), 4.80 (ABq, 2, 14 Hz), 5.11 (d, 1, 2 Hz), 5.30 (br s, 2), 7.1-7.6 (m, 2), 7.8-8.1 (m, 2).

Following substantially the same procedure as described above but substituting for the N,N'-diisopropyl-O-(m-methoxycarbonylbenzyl)-isourea used therein N,N'-diisopropyl-O-(p-(p-methoxybenzyloxy)carbonylbenzyl)-isourea, there was prepared p-(p-methoxybenzyloxy)carbonylbenzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate as an oil, NMR (CDCl₃): δ 2.02 (s, 3), 3.42 (ABq, 2, 19 Hz), 3.53 (s, 3), 3.73 (s, 3), 4.44 (d, 1, 2 Hz), 4.62 (d, 1, 2 Hz), 4.80 (ABq, 2, 13 Hz), 5.27 (ABq, 2, 13 Hz), 6.7-8.1 (m, 8).

Subsequently, following the same procedure as described above, 80 mg (0.15 mmols) of the above ester was oxidized to give 40 mg (47%) of p-(p-methoxybenzyloxy)carbonylbenzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-

ene-2-carboxylate-5,5-dioxide as an oil, NMR (CDCl₃);
δ 2.03 (s, 3), 3.53 (s, 3), 3.81 (ABq, 2, 18 Hz),
4.4-5.2 (m, 4), 5.26 (br s, 2), 6.7-8.1 (m, 8).

EXAMPLE 17

5

p-Hydroxycarbonylbenzyl 3-acetyloxymethyl-7α-methoxy-8-
-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
-5,5-dioxide

10

A solution of 35 mg (0.061 mmols) of p-(p-
methoxybenzyl)oxycarbonylbenzyl 3-acetyloxymethyl-7α-
methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-
carboxylate-5,5-dioxide in 0.8 ml of trifluoroacetic
acid was stirred with 0.2 ml of anisole at 0°C for 15
minutes. The reaction was concentrated in vacuo and
the residue was purified on a 1000 μ silica
preparative plate using 1% acetic acid in 50% ethyl
acetate-hexane as solvent to give 24 mg (87%) of
p-hydroxycarbonylbenzyl 3-acetyloxymethyl-7α-methoxy-
8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-
carboxylate-5,5-dioxide as a white solid, NMR
(CDCl₃): δ 2.02 (s, 3), 3.53 (s, 3), 3.83 (ABq, 2,
10 Hz), 4.67 (br s, 1), 4.83 (ABq, 2, 14 Hz), 5.12
(d, 1, 2 Hz), 5.33 (br s, 2), 7.68 (ABq, 4, 8 Hz).

20

25

EXAMPLE 18

Ethoxycarbonylmethyl 3-acetyloxymethyl-7α-methoxy-8-
oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-
5,5-dioxide

30

A solution of 1.0 g (3.5 mmols) of 3-acetyl-
oxymethyl-7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-
oct-2-ene-2-carboxylic acid and 4 ml of ethyl diazo-
acetate in 25 ml of methylene chloride was stirred

with 10 mg of rhodium (II) acetate dimer at 35°C for 30 minutes. The reaction was then concentrated in vacuo and the residue was purified by flash chromatography on silica gel eluting with 40-50% ethyl acetate-hexane. The fractions containing the product
5 are combined, evaporated and rechromatographed using 10% ethyl acetate-methylene chloride as eluant to give 250 mg (34%) of ethoxycarbonylmethyl 3-acetyloxy-methyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-
10 oct-2-ene-2-carboxylate, NMR (CDCl₃) 2.37 (t, 3, 7 Hz), 2.06 (s, 3), 3.43 (ABq, 2, 19 Hz), 3.53 (s, 3), 4.20 (q, 2, 7 Hz), 4.43 (d, 1, 2 Hz), 4.63 (d, 1, 2 Hz), 4.73 (ABq, 2, 16 Hz), 4.87 (ABq, 2, 13 Hz).

Following the same procedure as described in Example 1, Step C, 250 mg (0.67 mmols) of the
15 resulting ethoxycarbonylmethyl ester was oxidized to give 190 mg (69%) of ethoxycarbonylmethyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide, NMR (CDCl₃);
20 δ 1.29 (t, 3, 7 Hz), 2.07 (s, 3), 3.57 (s, 3), 3.89 (ABq, 2, 18 Hz), 4.18 (t, 2, 7 Hz), 4.74 (m, 3), 4.89 (ABq, 2, 14 Hz), 5.11 (d, 1, 2 Hz).

EXAMPLE 19

25 t-Butoxycarbonylmethyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

A solution of 500 mg (1.7 mmols) 3-acetyloxy-methyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-
30 oct-2-ene-2-carboxylic acid in 2 ml of N,N-dimethylacetamide was stirred with 300 mg (3.4 mmols) of sodium bicarbonate and 525 mg (3.4 mmoles) of t-butyl chloroacetate at room temperature for 16 hours. The

reaction was diluted with water and extracted with methylene chloride. The organic layer was washed with water and saturated aqueous sodium chloride solution dried over sodium sulfate and evaporated in vacuo. The residue was chromatographed on silica gel to give 20 mg of the t-butoxycarbonylmethyl ester as a mixture of 3-ene- and 2-ene-isomers which was oxidized directly, by following the procedure described in Example 1, Step C, to give 7 mg of t-butoxycarbonylmethyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide as an oil, NMR (CDCl₃); δ 1.48 (s, 9), 2.07 (s, 3), 3.51 (s, 3), 3.85 (AB, 2, 18 Hz), 4.5-5.0 (m, 5), 5.11 (d, 1, 2 Hz).

EXAMPLE 20

N-Benzyl-N-methyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide-5,5-dioxide

A solution of 2.0 g (7.0 mmol) of 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid in 10 ml of dioxane and 20 ml of acetone was stirred with 1.0 g (7.0 mmols) of isobutyl chloroformate and 600 μ l (7.0 mmols) of pyridine at -15°C. After 20 minutes 2.5 g (21 mmols) of N-methylbenzylamine was added and the reaction is stirred at -15°C for 1 hour, then allowed to warm to room temperature for 2 hours. The reaction was quenched with dilute hydrochloric acid and extracted into methylene chloride. The organic layer was washed with saturated aqueous sodium

bicarbonate solution and saturated aqueous sodium chloride solution, dried over sodium sulfate and evaporated. The residue was chromatographed on silica gel to give 280 mg (10%) of N-benzyl-N-methyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxamide, NMR(CDCl₃):

1.97 and 2.00 (s, 3), 2.83 and 2.90 (s, 3), 3.0 to 3.7 (m, 5), 4.3 to 4.8 (m, 6), 7.3 (br s, 5).

Following similar procedures as described in Example 1, Step C, 275 mg (.71 mmoles) of N-benzyl-N-methyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-formamide was oxidized to give 170 mg (57%) of N-benzyl-N-methyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide-5,5-dioxide, NMR(CDCl₃):

δ 1.97 and 2.00 (s, 3), 2.78 and 2.89 (s, 3), 3.4-4.1 (m, s), 4.3-4.8 (m, 5), 5.10 (d, 1, 2 Hz), 7.22 (br s, 5).

EXAMPLE 21

N-(t-Butoxycarbonyl)methyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide-5,5-dioxide

A solution of 1.0 g (3.4 mmoles) of 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid in 25 ml of methylene chloride was stirred with 1.1 g (5.1 mmole) of dicyclohexylcarbodiimide and 450 mg (3.4 mmols) of tert-butyl glycinate at room temperature for 4 hours. The reaction was concentrated in vacuo and the residue was eluted through a short column of silica gel using 50-60% ethyl acetate-hexane. The

fractions containing the resulting amide were combined and evaporated. The residue was further purified by chromatography on silica gel to give 230 mg (17%) of N-(t-butoxycarbonyl)methyl 3-acetyloxy-

5 methyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxamide, NMR(CDCl₃): δ 1.46 (s, 9), 2.07 (s, 3), 3.1-3.6 (m, 2), 3.50 (s, 3), 3.8-4.1 (m, 2), 4.47 (br s, 1), 4.63 (br s, 1), 4.87 (ABq, 2, 10 13 Hz), 7.4 (br s, 1).

Following similar procedures as described in Example 1, Step C, N-(t-butoxycarbonyl)methyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo-
15 [4.2.0]oct-2-ene-2-carboxamide was oxidized to give 60 mg of N-(t-butoxycarbonyl)methyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamide-5,5-dioxide, NMR(CDCl₃): δ 1.47 (s, 9), 2.07 (s, 3), 3.50 (s, 3), 3.5-4.1 (m, 4), 4.4-4.9 (m, 3), 5.07 (d, 1, 2 Hz), 7.3 (br s, 1).
20

EXAMPLE 22

Benzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

25 Trifluoroacetic acid (5 ml) was added to 316 mg (0.92 mm) of t-butyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide with cooling in an ice bath. After stirring for 0.5 hr at 0°C, trifluoroacetic acid was evaporated in vacuo. The residue was diluted with
30 dichloromethane and washed with cold water and brine. The dichloromethane solution was dried over

sodium sulfate. Crude 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid was obtained upon concentration of the filtrate. It was dissolved in 5 ml of tetrahydrofuran and N,N'-diisopropyl-O-benzyl-isourea (0.33 ml, 2.5 mmol) was added. After stirring for 80 hours the reaction mixture was poured into 7% sodium bicarbonate solution and extracted with ethyl acetate. The combined organic extract was washed with brine and dried over sodium sulfate. The concentrated filtrate was flash chromatographed using 50% ethyl acetate-hexane to yield 259 mg (77% yield) of benzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate as a mixture of δ 2 and δ 3 isomers. NMR(CDCl₃): δ 1.96 and 1.98 (s, 3), 3.4 (ABq, 0.8, 17 Hz), 3.43 (s, 1.8), 3.47 (s, 1.2), 4.2-5.3 (m, 6.6), 6.34 (bs, 0.6), 7-7.4 (m, 5).

Following substantially the same procedure as described in Example 1, Step C, 259 mg (0.69 mmol) of benzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate was oxidized to obtain 226 mg (80% yield) of benzyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide as a thick oil. NMR(CDCl₃): δ 2.01 (s, 3), 3.49 (s, 3), 3.8 (ABq, 2, 18 Hz), 4.6 (bs, 1), 4.78 (ABq, 2, 13 Hz), 5.07 (d, 1, 2 Hz), 5.17 (ABq, 2, 11 Hz), 7.1-7.4 (m, 5 H).

EXAMPLE 23

t-Butyl 3-acetyloxymethyl-7 α -ethyl-8-oxo-5-thia-1-
azabicyclo[4.2.0]oct-2-ene-2-carboxylate

A 2 liter, 3-necked round bottom flask fitted with 2 dropping funnels was charged with 200 ml THF and cooled to -78°C under N₂. One dropping funnel was charged with a solution of 6.33 g (15.3 mM) t-butyl 3-acetyloxymethyl-7-diazo-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate in 300 ml THF. The other funnel was charged with 32 ml 1M-triethylborane in THF, 1.2 ml H₂O, and 300 ml THF. The funnels were adjusted so their contents were added to the flask at 2.5 ml/min and the temperature of the reaction mixture maintained at -78°. After the addition, the cooling bath was removed and the reaction mixture allowed to warm. When the temperature reached -45°C, 6.67 ml 30% H₂O₂ was added. At -15°, the reaction mixture was washed with 300 ml brine, the organic layer diluted with 300 ml CH₂Cl₂, washed with 300 ml brine, dried over MgSO₄, filtered and stripped to give 7.46 g of yellow oil which was chromatographed on a flash column with CHCl₃/EtOAc (25:1) to yield 1.289 g (19%) t-butyl-3-acetyl-oxymethyl-7 α -ethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate a 4:1 mixture of the α and β isomers. NMR(CDCl₃): δ 1.08, t (J=8Hz), 3H; 1.50, s, 9H; 1.8, br, qt, 2H; 2.05, s, 3H; 3.1, brm, 1H; 3.4, d, 2H; 4.35, d (J=2Hz), 1H; 4.82, AB qt (J=12, 6Hz) 2H.

EXAMPLE 24

t-Butyl 3-acetyloxymethyl-7 α -methoxy-4-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5 β -oxide

5 Step A: Preparation of t-Butyl 3-acetyloxymethyl-7 α -methoxy-4-methylene-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5 β -oxide

10 Treatment of t-butyl 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5 β -oxide under Mannich conditions, (i.e., aq. formaldehyde and dimethylamine: HCl in DMF-dioxane) gave t-butyl 3-acetyloxymethyl-7 α -methoxy-4-methylene-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5 β -oxide as a foam, NMR (CDCl₃): δ 1.60 (9H, s), 2.03 (3H, s), 3.57 (3H, s), 4.50 (1H, d, J=1.5Hz), 4.68, 5.30, (2H, ABq, J=13.5Hz), 5.01 (1H, d, J=1.5Hz), 6.33 (2H, s).

20 Step B: Preparation of t-butyl 3-acetyloxymethyl-7 α -methoxy-4-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5 β -oxide

25 A solution of the 2-methylene derivative obtained in Step A (178 mg, 0.56 mmol) in ethyl acetate (5 ml) was hydrogenated at atmospheric pressure over an excess of 10% palladium on carbon. The catalyst was removed by filtration and the solvent removed by rotoevaporation to give a 1:1 mixture 4 α and 4 β -methyl isomers of t-butyl 3-acetyloxymethyl-7 α -methoxy-4-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5 β -oxide (170 mg). The isomers were separated by thick layer preparative chromatography on silica gel eluted with

50% ethyl acetate/hexane (5x). The assignment of the stereochemistry at C-4 was made by comparing the difference of the chemical shifts in the ^1H -NMR of the two isomers in CDCl_3 and C_6D_6 . The faster moving component was the 4 α -methyl isomer: oil; NMR (CDCl_3): δ 1.55 (3H, d, $J=7.0\text{Hz}$), 1.57 (9H, s), 2.10 (3H, s), 3.58 (3H, s), 3.88 (1H, q, $J=7.0\text{Hz}$), 4.36 (1H, d, $J=1.5\text{Hz}$), 4.67, 4.87 (2H, ABq, $J=13.5\text{Hz}$), 4.93 (1H, d, $J=1.5\text{Hz}$). The second component was the 4- β isomer: oil; NMR (CDCl_3): δ 1.58 (9H, s), 1.68 (3H, d, $J=7.0\text{Hz}$), 2.06 (3H, s), 3.56 (3H, s), 3.60 (1H, q, $J=7.0\text{Hz}$), 4.48 (1H, d, $J=1.5\text{Hz}$), 4.58, 5.15 (2H, ABq, $J=13.4\text{Hz}$), 4.91 (1H, d, $J=1.5\text{Hz}$).

EXAMPLE 25

t-Butyl 3-acetyloxymethyl-7 α -methoxy-4-methylene-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

To a stirred solution of t-butyl 3-acetyloxy-methyl-7 α -methoxy-4-methylene-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (180 mg, 0.48 mmol) in methylene chloride (2 ml) at 0°C was added m-chloroperbenzoic acid (109 mg, 0.53 mmol). After stirring the white suspension at room temperature overnight, methylene chloride (25 ml) was added and the solution successively washed with saturated sodium bicarbonate solution (3 x 10 ml) and water (2 x 10 ml). After drying over anhydrous sodium sulfate, the solvent was removed by rotoevaporation to give a whitish solid that was purified by flask column chromatography on silica gel eluted with 40%

ethyl acetate/hexanes to give 128 mg (69% yield) of t-butyl 3-acetyloxymethyl-7 α -methoxy-4-methylene-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide m.p. 155-157°C; ¹H-NMR (CDCl₃): δ 1.58 (9H, s), 2.03 (3H, s), 3.56 (3H, s), 4.66, 5.30 (2H, ABq, J=13.5Hz), 4.96 (1H, d, J=1.5 Hz), 5.21 (1H, d, J=1.5 Hz), 6.16 (1H, d, J=1.5Hz), 6.53 (1H, d, J=1.5Hz).

EXAMPLE 26

t-Butyl 3-acetyloxymethyl-7 α -methoxy-4 α -methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide

t-Butyl 3-acetyloxymethyl-7 α -methoxy-4 α -methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5 β -oxide was oxidized in the usual manner as described in Example 13 to afford t-butyl 3-acetyloxymethyl-7 α -methoxy-4 α -methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide as an oil. ¹H-NMR (CDCl₃): δ 1.55 (9H, s), 1.64 (3H, d, J=7Hz), 2.12 (3H, s), 3.56 (3H, s), 3.56 (1H, q, J=7Hz), 4.71 (1H, d, J=1.5Hz), 4.70, 4.86 (2H, ABq, J=13.5Hz), 5.16 (1H, d, J=1.5Hz); Field desorption mass spectrum, m/e 389.

Similarly, t-butyl 3-acetyloxymethyl-7 α -methoxy-4 β -methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5 β -oxide was oxidized to t-butyl 3-acetyloxymethyl-7 α -methoxy-4 β -methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide (an oil). ¹H-NMR (CDCl₃): δ 1.55 (9H, s), 1.56 (3H, d, J=7Hz), 2.05 (3H, s), 3.56 (3H, s), 3.96 (1H, q, J=7Hz), 4.56, 5.23 (2H, ABq, J=13.5Hz), 4.78 (1H, d, J=1.5Hz), 5.15 (1H, d, J=1.5Hz); field desorption mass spectrum, m/e 389.

EXAMPLE 27

t-Butyl 3-acetyloxymethyl-7 α -methoxy-4-phenylthio-
methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-
carboxylate-5,5-dioxide

5 Thiophenol (0.02 ml, 0.15 mmol) was added to
a stirred solution of t-butyl 3-acetyloxymethyl-7 α -
methoxy-4-methylene-8-oxo-5-thia-1-azabicyclo[4.2.0]-
oct-2-ene-2-carboxylate-5,5-dioxide (60 mg, 0.15
mmol) in methylene chloride (1 ml). After stirring
10 for 45 minutes, the solvent was removed by
rotoevaporation and the residue purified by thick
layer preparative chromatography on silica gel eluted
with 30% ethyl acetate/hexanes to afford t-butyl
3-acetyloxymethyl-7 α -methoxy-4-phenylthiomethyl-8-
15 oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-
5,5-dioxide as a pale yellow oil (38 mg, 50%
yield): $^1\text{H-NMR}(\text{CDCl}_3)$: δ 1.53 (9H, s), 2.00
(3H, s), 3.33 (1H, dd, J=15 and 9Hz), 3.51 (3H, s),
3.57 (1H, d, J=15Hz), 3.80 (1H, m), 4.21, 4.40 (2H,
ABq, J=12Hz), 5.10 (2H, br s); field desorption mass
20 spectrum, m/e 497.

EXAMPLE 28

t-Butyl 7 α -methoxy-3-[[[(1,2,5,6-tetrahydro-5,6-dioxo-
2-methyl-as-triazin-3-yl)thio]methyl]-8-oxo-5-thia-
25 1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate-5,5-di-
oxide

A solution of 32 mg (0.2 mmol) of 1,2,5,6-
tetrahydro-5,6-dioxo-3-mercapto-2-methyl-astriazine
in 1 ml of water was prepared by adding 35 mg (0.42
30 mmol) of NaHCO_3 . A solution of 70 mg (0.2 mmol) of
t-butyl 3-chloromethyl-7 α -methoxy-8-oxo-5-thia-

1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate-5,5-dioxide in 2 ml of acetone was added. After stirring the reaction mixture overnight it was concentrated in vacuo. The residue was partitioned between 7% NaHCO₃ and ether. The ether layer was extracted with 7% NaHCO₃ solution. The combined aqueous layer was washed with ether. The aqueous layer was acidified to pH 2 in the presence of ethyl acetate using concentrated HCl. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated NaCl and dried. The filtrate was concentrated and the residue was crystallized from ethyl acetate and ether. t-Butyl 7 α -methoxy-3-[[[(1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-as-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate-5,5-dioxide (76 mg, 80% yield) was obtained as a light yellow solid.

HNMR (CDCl₃): δ 1.58 (s, 9H), 3.58 (s, 3), 3.77 (s, 3H), 3.8-4.5 (m, 5H), 4.74 (bs, 1H), 5.18 (bs, 1H).

EXAMPLE 29

t-Butyl 7 α -methoxy-3-[[[(1-carboxymethyl-tetrazol-5-yl)-thio[methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate-5,5-dioxide

A solution of 32 mg (0.2 mmol) of 1-carboxymethyl-5-mercaptotetrazole in 1 ml of water was prepared by adding 35 mg (0.42 mmol) of NaHCO₃. A solution of 70 mg (0.2 mmol) of t-butyl 3-chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate-5,5-dioxide in 2 ml of

acetone was added. After stirring the reaction mixture overnight it was concentrated in vacuo. The residue was partitioned between 7% NaHCO₃ solution and ether. The aqueous layer was neutralized with concentrated HCl in the presence of ethyl acetate. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated NaCl and dried. The filtrate was concentrated and the residue was triturated with ether to obtain 70 mg (73% yield) of t-Butyl 7 α -methoxy-3[[(1-carboxymethyl-tetrazol-5-yl)-thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate-5,5-dioxide as a white powder. HNMR (CDCl₃): δ 1.58 (s, 9H), 3.56 (s, 3H), 3.87 (d, J=17 Hz, 1H), 4.05 (d, J=13 Hz, 1H), 4.2 (d, J=17 Hz, 1H), 4.6 (d, J=13 Hz, 1H), 4.75 (s, 1H), 5.16 (ABq, J=16 Hz, 2H), 5.16 (bs, 1H), 6.9 (broad, 1H).

EXAMPLE 30

N-Methyl-N-t-butoxycarbonylmethyl-3-chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate-5,5-dioxide

To a solution of 2.6 g (5.8 mmol) of N-methyl-N-t-butoxycarbonylmethyl-3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxamide-5,5-dioxide in 13 ml of 2-propanol was added 1.95 ml (6.5 mmol) of titanium (IV) isopropoxide. The solution was heated in a 55° bath. After 1.5 hours the orange reaction mixture was diluted with ethyl acetate and poured into water. The layers were separated and the aqueous layer was

extracted with ethyl acetate. The combined organic layer was washed with water, saturated NaCl and dried. The filtrate was concentrated in vacuo to obtain 2.06 g of residue.

5 The residue was dissolved in 20 ml of tetrahydrofuran and 1.75 ml of pyridine. The solution was cooled in an ice-bath and 0.58 ml (7.4 mmol) of thionyl chloride was added. After stirring for 20 minutes the dark reaction mixture was diluted with ethyl acetate and poured into water. The layers
10 were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with 7% NaHCO₃, water, 1.2 N HCl, saturated NaCl and dried. The filtrate was concentrated. The residue was chromatographed on a flash column using
15 60% ethyl acetate-hexane to obtain 710 mg (29% yield) of N-methyl-N-t-butoxycarbonylmethyl-3-chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxamide-5,5-dioxide.

20 ¹H NMR (CDCl₃): δ 1.48 and 1.52 (2s, 9H), 3.1 and 3.12 (2s, 3H), 3.39 and 3.57 (2s, 3H), 3.6-4.8 (m, 6H), 4.92 (bs, 1H), 5.25 (d, J=2 Hz, 1H).

EXAMPLE 31

25 N-Methyl-N-t-butoxycarbonylmethyl-7 α -methoxy-3[[1,2,5,6-tetrahydro-5,6-dioxo-2-methyl-as-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxamide-5,5-dioxide

30 A solution of 53 mg (0.33 mmol) of 1,2,5,6-tetrahydro-5,6-dioxo-3-mercapto-2-methyl-as-triazine in 1 ml of water was prepared by adding 57 mg (0.68 mmol) of NaHCO₃. A solution of 140 mg (0.33 mmol) of

N-methyl-N-t-butoxycarbonylmethyl-3-chloromethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxamide-5,5-dioxide in 2 ml of water was added. After stirring the reaction mixture overnight it was concentrated in vacuo. The residue was
5 partitioned between 1% NaHCO₃ and ether. The aqueous layer was acidified with concentrated HCl in the presence of ethyl acetate. The layers were separated and the aqueous layer was extracted with ethyl
10 acetate. The combined organic layer was washed with saturated NaCl and dried. The filtrate was concentrated and the residue was triturated with ether to obtain 110 mg (60% yield) of N-methyl-N-t-butoxycarbonylmethyl-7 α -methoxy-3[[1,2,
15 5,6-tetrahydro-5,6-dioxo-2-methyl-as-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxamide-5,5-dioxide as a white solid.
¹HNMR (Acetone-d₆): δ 1.48 (s, 9H), 3.06 (broad, 1H), 3.15 (s, 3H), 3.55 and 3.56 (2s, 3H),
20 3.73 and 3.75 (2s, 3H), 3.9-4.5 (m, 6H), 5.26 (s, 1H), 5.38 (s, 1H).

EXAMPLE 32

3-Acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-aza-bicyclo
25 [4.2.0]-oct-2-ene-2-morpholino carboxamide

Step A: Preparation of 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-aza-bicyclo[4.2.0]-oct-3-ene-2-morpholino carboxamide

30 To a solution of 3-acetoxymethyl-7-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid (5.0 g) in dioxane (50 ml) were added

N-hydroxysuccinimide (2.4 g) and dicyclohexyl carbodiimide (5.4 g). After stirring for 0.5 hour, the reaction was cooled to 5°C and triethylamine (4 mL) and, after another 15 minutes, morpholine (3.0 g) were added. After 1.5 hours stirring at room temperature, the reaction was diluted with diethyl ether, filtered and washed with water containing 40 mL of 2N HCl. The aqueous layer was extracted with ethyl acetate (3x) and each layer was consecutively washed with water and brine. The combined organic layers were dried over sodium sulfate and evaporated and the residue flash chromatographed (60 to 70% ethyl acetate/hexanes) to give 2.0 g of 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-aza-bicyclo[4.2.0]-oct-3-ene-2-morpholino carboxamide. NMR (CDCl₃): δ 2.08 (s, 3H), 3.54 (s, 3H), 3.6-3.9 (m, 8H), 4.57 (AB quartet, 2H), 4.60 (br s, 1H), 4.92 (s, 1H), 5.32 (br s, 1H), 6.54 (br s, 1H).

Step B: Preparation of 3-Acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-morpholino carboxamide

A solution of 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-3-ene-2-morpholino carboxamide (2.0 g) and *m*-chloroperbenzoic acid (3.4 g) in methylene chloride (100 mL) was stirred at room temperature for 16 hours. Amberlyst A-21 resin (17 g) was then added and, after 0.5 hours, the mixture was filtered and evaporated. Flash chromatography (70% ethyl acetate/hexanes) of the residue afforded 1.85 g of 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-morpholino carboxamide-5,5-dioxide.

NMR (CDCl₃): δ 2.13 (s, 3H), 3.4-4.1 (m, 10H), 3.58 (s, 3H), 4.57 (ABq, 2H), 4.82 (br s, 1H), 5.23 (d, 1H, J=1Hz).

EXAMPLE 33

5 3-Acetoxymethyl-7α-methoxy-8-oxo-5-thia-1-azabicyclo
[4.2.0]-oct-2-ene-2-(2-(S)-carboxypyrrolidinocarbox-
amide)-5,5-dioxide

10 Step A: Preparation of t-butyl 3-acetoxymethyl-
7α-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-3-
ene-2-(2-(S)-carboxypyrrolidinocarboxamide)

15 To a solution of 3-acetoxymethyl-7-methoxy-
2-cephem-4-carboxylic acid (7.4 g) in dioxane (100
ml) was added N-hydroxysuccinimide (3.5 g) followed
by dicyclohexylcarbodiimide (7.9 g). After 0.5 hour
at room temperature, the reaction was cooled to 5°C
and triethylamine (7.1 mL) was added. After 15
minutes, L-proline t-butyl ester (6.6 g) was added.
20 After 1.5 hour at room temperature, the reaction was
diluted with ether, filtered and washed with dilute
hydrochloric acid. The aqueous layer was extracted
with another 2 portions of ethyl acetate and each
organic layer was washed with water 2% sodium
bicarbonate solution and brine. The combined organic
25 layers were dried over sodium sulfate and
evaporated. The residue on flash chromatography (40%
ethyl acetate/hexanes) gave 0.80 g of t-butyl
3-acetoxymethyl-7α-methoxy-8-oxo-5-thia-1-azabicyclo
[4.2.0]-oct-3-ene-2-(2-(S)-carboxypyrrolidinocarbox-
30 amide). NMR (CDCl₃): δ 1.45 and 1.52 (2s, 9H), 2.08

and 2.08 (2s, 3H), 1.8-2.4 (m, 4H), 3.52 and 3.54 (2s, 3H), 3.75 (m, 1H), 4.05 (m, 1H), 4.4 (m, 1H), 4.60 (ABq, 2H), 4.66 (br s, 1H), 4.96 (br s, 1H), 5.20 (br s, 1H), 6.44 and 6.46 (2 br s, 1H).

5 The above sodium bicarbonate wash was acidified with 2N hydrochloric acid in the presence of ethyl acetate and the ethyl acetate layer was washed with brine, dried over sodium sulfate and evaporated to give 3.4 g of essentially pure
10 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-3-ene-2-carboxylic acid.

NMR (CDCl₃): δ 2.10 (s, 3H), 3.58 (s, 3H), 4.50 (s, 1H), 4.55 (ABq, 2H), 5.06 (s, 1H), 5.10 (br s, 1H), 6.51 (br s, 1H), 7.06 (br s, 1H).

15 Step A: (Alternate) Preparation of t-butyl 3-acetoxy-methyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-3-ene-2-(2-(S)-carboxypyrrolidinocarboxamide

20 To a solution of 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-3-ene-2-carboxylic acid (0.5 g) in dioxane (20 mL) at 5°C was added dicyclohexylcarbodiimide (0.71 g), followed after 10 minutes by slow addition of L-proline
25 t-butyl ester (0.30 g) in 5 mL of dioxane over 5 minutes. The reaction was stirred at room temperature for 24 hours before being diluted with ether, filtered and washed with dilute hydrochloric acid. The aqueous layer was extracted with 3
30 portions of ethyl acetate and each organic layer consecutively was washed with water and brine. The combined organic layers were dried over sodium

sulfate and evaporated. The residue on flash chromatography (40% ethyl acetate/hexanes) afforded 450 mg of t-butyl 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-3-ene-2-(2-(S)-carboxypyrrolidinocarboxamide). NMR was same as above.

Step B: Preparation of t-butyl 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-(2-(S)-carboxypyrrolidinocarboxamide)-5,5-dioxide

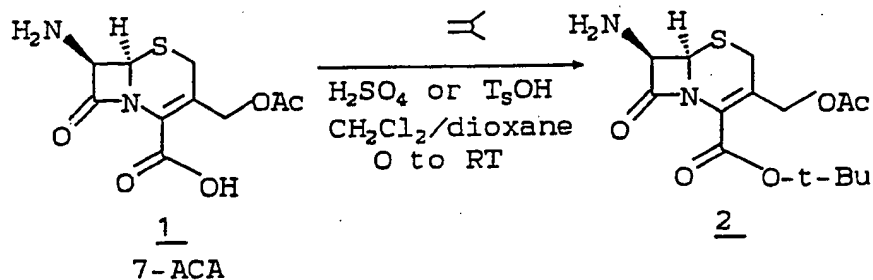
A solution of t-butyl 3-acetoxymethyl-7 α -methoxy-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-3-ene-2-(2-(S)-carboxypyrrolidinocarboxamide) (3.1 g) and m-chloroperbenzoic acid (4.7 g) in methylene chloride (150 mL) was stirred at room temperature for 16 hours. The reaction was then poured into a solution of sodium bicarbonate and sodium sulfite and the layers separated. The organic layer was washed with brine, dried over sodium sulfate and evaporated after addition of 5 drops of pyridine. The residue on flash chromatography (40-60% ethyl acetate/hexanes) afforded 2.8 g of 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-(2-(S)-carboxypyrrolidinocarboxamide)-5,5-dioxide t-butyl ester. NMR (CDCl₃): δ 1.40 and 1.44 (2s, 9H), 2.01 and 2.03 (2s, 3H), 1.8-2.4 (m, 4H), 3.4-3.7 (m, 2H), 3.49 and 3.52 (2s, 3H), 3.80 (ABq, 2H), 4.17 and 4.38 (2 dd, 1H, J= 8 Hz, J=2 Hz), 4.5-4.8 (2 ABq, 2H), 4.80 and 4.85 (2 br s, 1H), 4.84 (2d, 1H, J=2Hz).

Step C: Preparation of 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-(2-(S)-carboxypyrrolidinocarboxamide)-5,5-dioxide

t-Butyl 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-(2-(S)-carboxypyrrolidinocarboxamide)-5,5-dioxane (400 mg) was taken up in 1 mL of anisole and 15 mL of TFA (precooled to 0°C) and stirred in an ice bath for 1/2 hour. Most of the TFA/anisole was removed in vacuo and then under a stream of N₂. The residue was eluted on 2 x 2000 μ m silica prep plates (1% HOAc in ethyl acetate) to give 250 mg of 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-(2-(S)-carboxypyrrolidinecarboxamide)-5,5-dioxide. NMR (CDCl₃): δ 2.08 and 2.11 (2s, 3H), 1.8-2.4 (m, 4H), 3.56 and 3.60 (2s, 3H), 3.4-4.0 (m, 2H), 3.90 (ABq, 2H), 4.4-5.0 (m, 3H), 4.90 (br s, 1H), 4.26 (br s, 1H).

EXAMPLE 34

Step A: Preparation of t-butyl 3-acetyloxymethyl 7B-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (t-butyl 7-ACA, 2)



*t-Bu can be substituted by another protecting group, e.g., C₁₋₆ alkyl; benzhydryl, benzyl and the like.

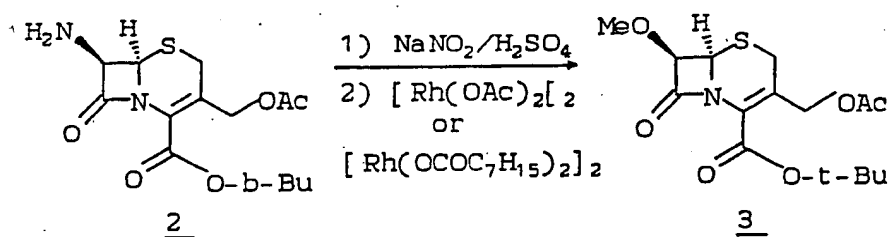
5 A suspension of tosic acid monohydrate (500 gms, 2.6 moles) in toluene (2L) was dehydrated by refluxing under a Dean-Stark trap for 40 hrs. At this time all of the tosic acid had gone into solution and a total of 57 mL of water had been collected. The toluene was then removed in vacuo to afford a colored, solid mass.

10 The dehydrated tosic acid was taken up in methylene chloride (2500 mL) and transferred to a 5-L 3-necked round bottom flask which had been fitted with a mechanical stirrer, N₂ and gas inlet tubes and a dry ice condenser. Solid 7-ACA (1) (355 gms, 1.3 moles) was slowly added at a rate to achieve solution of the 7-ACA without formation of a gummy mass. The solution was then cooled to 10-15°C and isobutylene (1000 mL) was distilled into the reaction mixture over 2-1/2 hrs. Note: Rapid addition of the isobutylene causes formation of a gummy precipitate. 20 The reaction mixture was stirred overnight at room temperature.

25 The reaction was slowly quenched with vigorous stirring into a 5-gallon carboy containing a solution of sodium bicarbonate (500 gms) in ice water (6L). The organic layer was separated using suction and washed with water and brine. The aqueous layers were sequentially back-extracted with two portions of methylene chloride (500 mL) and the combined layers were dried over sodium sulfate. Most of the methylene 30

chloride was removed in vacuo until the product began to solidify. At this time cyclohexane (1L) was added and the precipitate was triturated to break up the clumps. The product was collected by filtration, washed with cyclohexane and dried overnight by pulling air through the filter cake. The yield of t-butyl 7-ACA (2) was 360 grms (80%) as an off-white solid. R_f (Et₂O) = 0.3 - 0.5.

Step B: Preparation of t-butyl-3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylate (3)



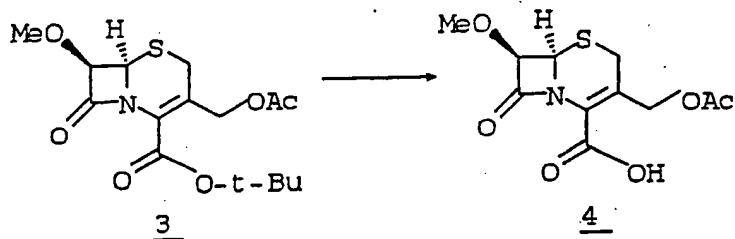
The diazotization reaction was run in two 50 gm batches and then combined for the work-up and second step.

To duplicate solutions of t-butyl 7-ACA 2 (2 x 50 gms, 0.30 moles) in methylene chloride (2 x 600 mL) was added sodium nitrite (2 x 11.4 g, 0.33 moles) dissolved in water (2 x 500 mL). The mixtures were cooled in an ice bath and to each was added 2N H₂SO₄

(2 x 114 mL, 0.450 moles) in portions over five minutes and the reactions were stirred for 45 minutes at 0°C. They were then combined and the organic layer was separated (some problems with emulsions) and washed with water and brine. The aqueous layers were back-extracted with methylene chloride and the organic layers combined, dried over sodium sulfate and filtered.

To the above methylene chloride solution was added at room temperature methanol (800 mL) and, while vigorously stirring, rhodium acetate dimer (0.64 gms). Vigorous nitrogen evolution occurred and after 1/2 hr the reaction was filtered through celite and concentrated in vacuo at low temperature. The residue was taken up in ether and washed with water and brine to remove any residual methanol. The aqueous layers were back-extracted with ether and the organic layers were combined, dried over sodium sulfate and evaporated in vacuo. The residue was purified by preparative HPLC in two portions using 15% EtOAc/hexane as eluent. The yield of 3 was 25-30 gms (25%) as a yellow oil which slowly crystallized on standing. R_f (25% EtOAc/hex) = 0.50. The product 3 was the first major component on TLC.

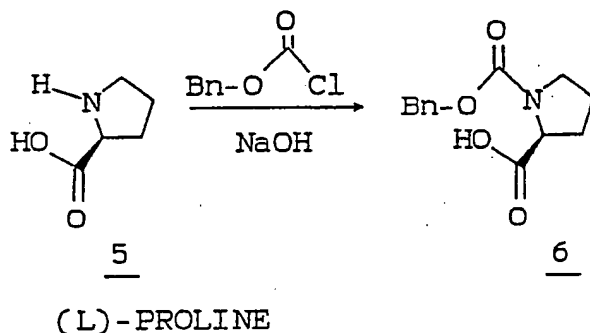
Step C: Preparation of 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid (4)



10 To a solution of TFA (75 mL) and anisole (5
mL) at 0° was added the t-butyl sulfide 3 (18 gms) as
a solid or in a minimum amount of methylene chloride.
The reaction was stirred at 0° for 1 hr or until it
was judged nearly complete by TLC (Note: The
15 reaction never seems to go to completion). The
solution was then poured into a mixture of ice water
(200 mL) and methylene chloride (200 mL) and the
organic layer was separated and washed twice with
water (100 mL). Each aqueous layer was sequentially
20 back extracted with an additional three portions of
methylene chloride (200 mL). The combined methylene
chloride layers were extracted twice with water
containing enough sodium bicarbonate solution to
maintain the pH at 7-8 and each extract was washed
25 with methylene chloride. The combined aqueous layers
were acidified with 2N hydrochloric acid in the
presence of ethyl acetate. The layers were separated
and the organic layer was washed with brine. The
aqueous layers were back extracted with three
30 portions of ethyl acetate (50 mL) and these were
combined, dried over sodium sulfate and evaporated.

The crude product 4 was usually a dark, sticky foam and was used as obtained as soon as possible. The yield of 4 was typically 12-14 gms (80-90%).

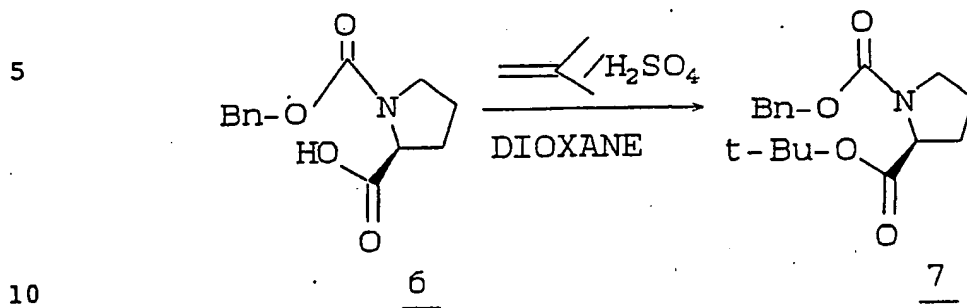
Step D: Preparation of N-benzoxycarbonyl-L-Proline
(6)



L-Proline 5 (100 gms, .87 moles) was dissolved in 2N sodium hydroxide (430 mL) and cooled in an ice bath. A further 220 mL of 4N sodium hydroxide was added simultaneously with benzyl chloroformate (155 gms, .90 moles) over 2 hrs. The reaction was then allowed to warm to room temperature overnight.

The reaction was washed twice with ether (500 mL) and the aqueous layer acidified with concentrated hydrochloric acid and extracted twice with ethyl acetate (500 mL). The combined organic layers were washed with brine and dried over sodium sulfate. Evaporation in vacuo gave 268 gms of crude 6 as a thick oil. This was used directly in the next reaction.

Step E: Preparation of t-butyl N-benzoxycarbonyl-
pyrrolidine-2-carboxylate (7)



*t-Bu can be substituted by a protecting group such as C₁₋₆ alkyl, benzyl, benzhydryl and the like.

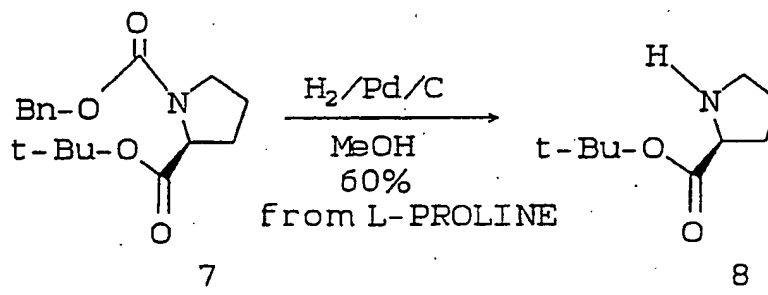
15 The above product 6 was taken up in dioxane (1400 mL) and placed in two 2-L pressure bottles. To each bottle was added conc. sulfuric acid (30 mL) and condensed (dry ice bath) isobutylene (350 mL).

20 The bottles were stoppered, fastened with wire and the reactions were stirred at room temperature overnight. The reactions became homogeneous after approximately 1 hr.

25 The solutions were cooled (until dioxane started to freeze), carefully vented and poured into a carboy containing a solution of sodium bicarbonate (250 gms) in ice water (4 L) and ether (2L). The layers were separated and the ether was washed with water and brine. The aqueous layers were back-extracted with ether and the ether layers were combined and dried over sodium sulfate. Evaporation in vacuo gave 185 gms of crude 7 as a clear oil.

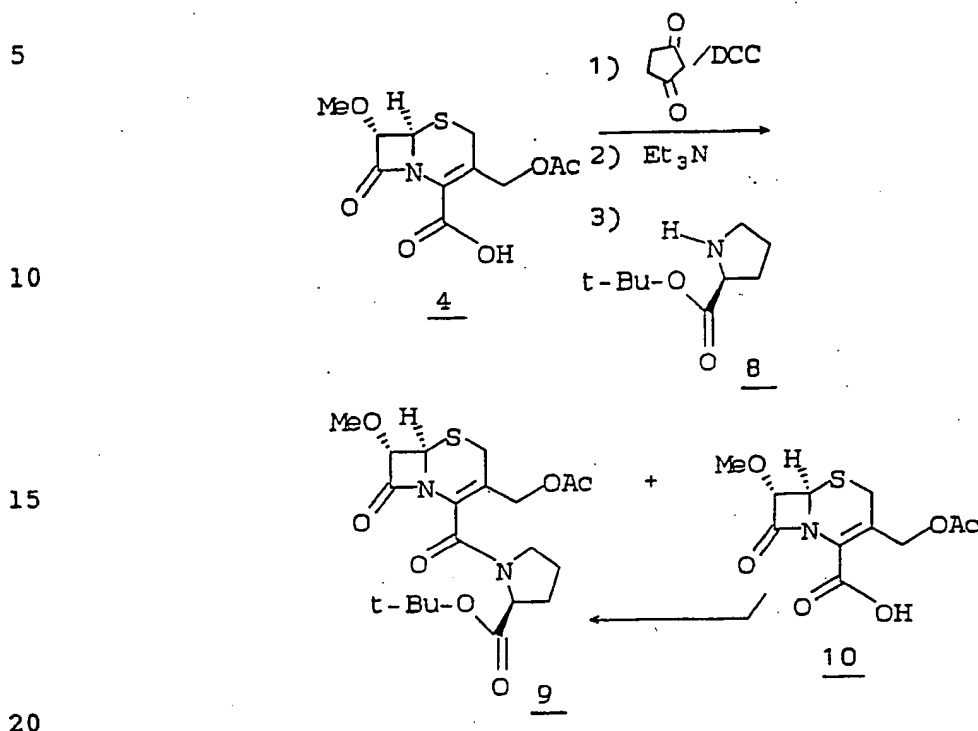
30 This was used directly in the next step.

Step F: Preparation of t-butyl pyrrolidine-2-
carboxylate (8)



The above oil 7 was taken up in methanol (1500 mL) and hydrogenated over 10% pd/C (8 gms) for 20 hrs. (left overnight for convenience). The reaction was filtered and concentrated in vacuo without heating. The residue was distilled at 55-65° C/ 1 torr to give 85 gms (60% overall from L-proline) of t-butyl L-proline (8).

Step G: Preparation of 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-(2-(s)-carboxypyrrolidinecarboxamide) (9)



To an ice bath cooled solution of the crude acid 4 (11.5 gms, 40 mmoles) in dioxane (100 mL) are sequentially added N-hydroxysuccinimide (5.7 gms, 50 mmole) and DCC (12.4 gms, 60 mmoles). The reaction was then stirred under nitrogen at room temperature for 1/2 hr during which time a thick precipitate formed. The reaction was again cooled in an ice bath before dry triethylamine (5.5 mL, 40 mmoles) was added and the stirring was continued for another 1/2 hr. Finally, t-butyl L-proline 8 (14 gms, 80 mmole) was added all at once. After a further 2 hrs at room

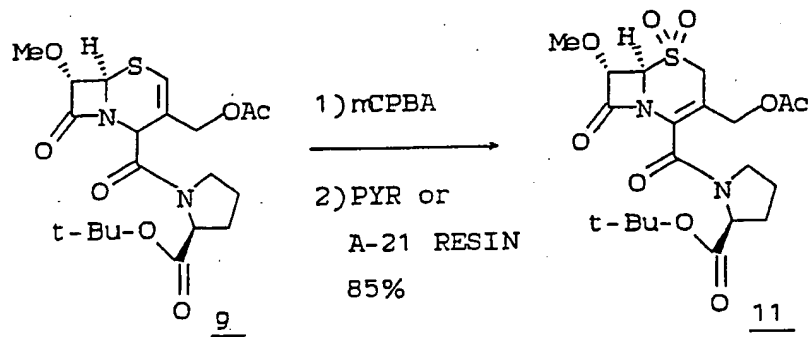
temperature, the reaction was diluted with ether (200 mL), filtered, and quenched into ice water (200 mL) containing 60 mL of 2N hydrochloric acid. The layers were separated and the organic layer was washed with water, sodium bicarbonate solution and brine. Each aqueous layer was back extracted with another 100 mL of ether. The organic layers were combined, dried over sodium sulfate and evaporated in vacuo. The product 9 was purified by preparative HPLC (45%) EtOAc/hexanes to give 10.5 gms (60%) of 9 as a slightly colored oil. The product 9 was usually accompanied by a small amount of Δ^3 product as well as some DCC by-product.

The above sodium bicarbonate wash of was acidified in the presence of EtOAc to a pH of 1-2 layers were separated. The aqueous layer was re-extracted with another two portions of ethyl acetate and the organic layers were combined, dried over sodium sulfate and evaporated to give 0.5 - 2.0 gms of recovered Δ^2 acid 10. This was readily recycled similar to the above reaction to obtain 9.

Step H: Preparation of t-butyl 3-acetoxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0.]oct-2-ene-2-(2-(s)-carboxypyrrolidine carboxamide)-5,5-dioxide (11)

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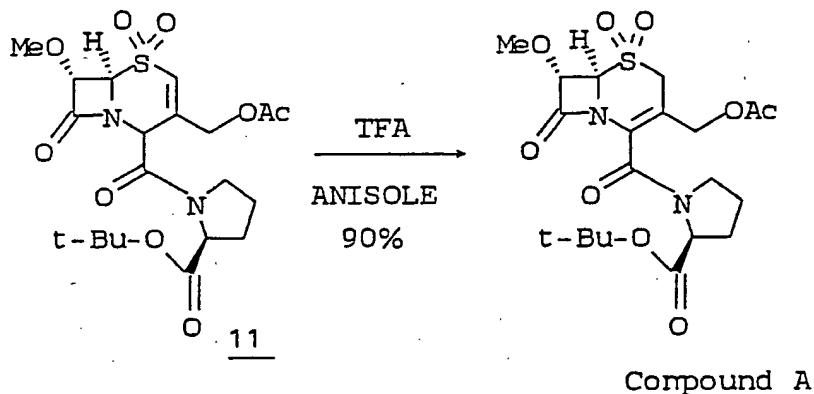
To a solution of Δ^2 -sulfide 9 (10.5 gms, 24 mmole) in methylene chloride (700 mL) (Note: The reaction should be kept dilute to avoid any problem with t-Bu ester loss) was added 85% m-chloroperbenzoic acid (14.7 gms, 72 mmole). The solution was stirred at room temperature overnight. The reaction was then quenched into a mixture of sodium bicarbonate and excess sodium sulfite. The layers were separated and the organic layer was washed with brine containing a few milliliters of saturated sodium sulfite solution. The aqueous layers were back extracted with methylene chloride and the organic layers were combined and dried over sodium sulfate. Pyridine (5 drops) or amberlyst A-21 resin (5-10 gms) were added and the mixture stirred for 1/2 hr in order to completely isomerize the product to Δ^3 . Filtration (for A-21

resin) and evaporation gave a crude residue which was purified by preparative HPLC (50% EtOAc/hexanes) to give 9.5 gms (85%) of 11 as a white foam.

5 Step I: Preparation of 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-(2-(s)-carboxypyrrolidinecarboxamide)-5,5-dioxide (compound A)

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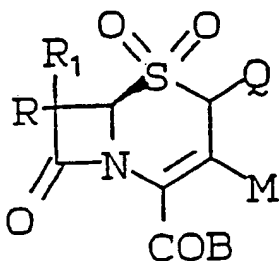
To an ice bath cooled solution of TFA (75 mL) and anisole (5 mL) was added the t-butyl sulfone 11 (8.0 gms). After stirring for 1 hr at 0°C, the reaction was evaporated in vacuo without much heating. The residue was taken up in methylene chloride and re-evaporated to remove most of the TFA. The remaining volatiles were blown off in a stream of nitrogen and the product was precipitated and triturated with ether and filtered. The product was redissolved in a minimum amount of methylene chloride and reprecipitated with ether to give 6.4 gms of off-white solid. The combined mother liquors

were evaporated and the residue was flash chromatographed eluting with a solvent gradient of 60% EtOAc/hexane to 80% EtOAc/hexane and finally 1% HOAc/EtOAc to give 950 mg of pure compound A after evaporation of 50 mL of toluene to remove any residual acetic acid.

The 6.4 gms were flash chromatographed as above in 3 portions, obtaining 1.86 gms of compound A from a 2.0 gm portion. NMR (CDCl₃): δ 2.08 and 2.11 (2s, 3H); 1.8-2.4 (m, 4H); 3.56 and 3.6 (2s, 3H); 3.4-4.0 (m, 2H); 3.90 (ABq, 2H); 4.4-5.0 (m, 3H); 4.90 (brs, 1H); 4.26 (brs, 1H).

WHAT IS CLAIMED IS:

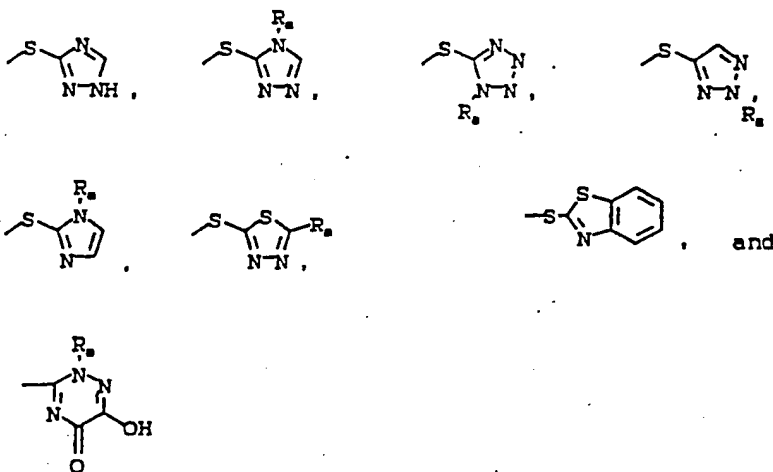
1. A pharmaceutical composition comprising: a pharmaceutical carrier, a therapeutically effective amount of compound selected from the group consisting of epsilon-aminocaproic acid, heparin, trasylol, prednisolone, cytosine arabinoside, b-mercaptopurine, cytarabine, an anthracycline and a vitamin A derivative; and a therapeutically effective amount of compound of formula I



wherein R is hydrogen, chlorine or fluorine;
M is:

- (1) trifluoromethyl;
- (2) chloro or fluoro;
- (3) -COOH;
- (4) -CHO; or
- (5) -CH₂A wherein A represents
 - (a) hydrogen;
 - (b) halo;
 - (c) ZR₅ wherein Z is oxygen or sulfur and R₅ is

- 5 (1) straight or branched chain C_{1-6} alkyl;
- (2) H;
- (3) straight or branched chain C_{2-6} alkenyl; or
- (4) straight or branched chain C_{1-6} alkynyl;
- 10 (d) acyloxy wherein acyl is C_{2-6} alkanoyl, phenylcarbonyl, benzyloxycarbonyl, succinoyl, carbamoyl, $N-C_{1-6}$ alkyl or N,N -di C_{1-6} alkyl carbamoyl, thiocarbamoyl, or $N-C_{1-6}$ alkyl or N,N -di C_{1-6} alkyl- thiocarbamoyl;
- (e) phenoxy;
- 15 (f) benzyloxy;
- (g) phenylthio or benzylthio;
- (h) acylthio wherein the acyl group is as previously defined;
- (i) $-S-C-OR_a$ or $-S-C-OR_a$ wherein R_a
- 20 $\begin{matrix} S & O \\ & | & | \\ & CH_2COOH \end{matrix}$ represents C_{1-6} alkyl, phenyl or CH_2COOH ;
- (j) a substituted or unsubstituted amino or amido group selected from a group consisting of amino, $N-C_{1-6}$ alkylamino, N,N -di C_{1-6} alkylamino;
- 25 (k) R_a-SO- ; or
- (l) R_a-SO_2- ;
- 30 (m) a substituent selected from the group consisting of



the above groups (a) to (1) can be unsubstituted or substituted with one or more radicals selected from a group consisting of C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, cyano, carboxy, carbamoyl, azido, sulfo, amino, C₁₋₆ alkylamino, diC₁₋₆ alkylamino, halo C₁₋₆ alkyl, N-C₁₋₆ alkyl-guanidino, guanido C₁₋₆ alkyl, sulfomyl and N-C₁₋₆ alkyl-sulfamyl;

(6) -CH=CH-R_a;

R₁ is

(a) hydrogen;

(b) hydroxy;

(c) mercapto;

(d) OR'₁ wherein R'₁ is a hydrocarbyl group selected from a group consisting of straight or branched chain C₁₋₆ alkyl, C₃₋₆ alkenyl, C₃₋₆ alkynyl, phenyl and benzyl, these hydrocarbyl groups may be unsubstituted or substituted with one or more radicals which is hydroxy, halo, nitro, amino, carboxy or thio;

(e) $-SR'_1$ wherein R'_1 is as previously defined;

(f) a hydrocarbyl group selected from a group consisting of

- 5
- (1) straight or branched C_{1-6} alkyl;
 - (2) straight or branched C_{2-6} alkenyl;
 - (3) straight or branched C_{2-6} alkynyl;
 - (4) phenyl C_{1-6} alkyl;
 - (5) C_{3-6} cycloalkyl;
 - (6) phenyl;
 - 10 (7) $-CF_3$;

the above groups (1) to (6) can be unsubstituted or substituted with one or more radicals selected from a group consisting of halo, hydroxy, C_{1-6} alkoxy, amino, nitro, sulfonyl, sulfamoyl, acyloxy, carbamoyloxy, carboxy, carboxamido and $N-C_{1-6}$ alkyl or $N,N-C_{1-6}$ alkylcarboxamido;

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(g) cyano;

(h) $\begin{array}{c} X \\ || \\ -C-R'' \end{array}$ wherein X is oxygen or sulfur and R'' is

20

- (1) hydrogen;
- (2) hydroxy;
- (3) mecapto;
- (4) amine;
- 25 (5) N-alkyl or N,N-dialkylamine;
- (6) C_{1-6} alkyl;
- (7) phenyl;
- (8) benzyl;
- (9) benzyloxy;
- 30 (10) C_{1-6} alkyloxy;
- (11) phenoxy;

(12) C₁₋₆ alkylthio; or

(13) phenylthio;

(i) halo;

(j) $\begin{array}{c} \text{O} \\ \parallel \\ -\text{P}-\text{Y}-\text{Z}' \end{array}$ where Y' and Z' independently

are OH, or -OC₁₋₆ alkyl;

(k) $\begin{array}{c} \text{O} \\ \parallel \\ -\text{O}-\text{C}-\text{CH}_3 \end{array}$

(l) -SO₂NH₂;

(m) -SO₂R₂ where R₂ is C₁₋₆ alkyl, halo C₁₋₆ alkyl, phenyl, or benzyl which can be unsubstituted or substituted as previously defined;

(n) -SO₂NR₃R₄ where R₃ and R₄ independently represent H or C₁₋₆ alkyl;

B is OB₁ or NB₂B₃ wherein B₁ and B₂ independently are:

(a) straight or branched chain alkyl having from 1 to 20 carbon atoms;

(b) aryl having from 6 to 10 carbon atoms;

(c) cycloalkyl having from 3 to 8 carbon atoms;

(d) alkenyl having from 2 to 12 carbon atoms;

(e) cycloalkenyl having from 5 to 8 carbon atoms;

(f) alkynyl having from 2 to 12 carbon atoms;

(g) alkoxy alkyl having from 2 to 12 carbon atoms;

(h) aralkyl, alkaryl, aralkenyl, aralkynyl, alkenylaryl or alkynylaryl wherein alkyl, alkenyl and alkynyl are as previously defined, and the aryl group is selected from the group consisting of

(1) phenyl,

- (2) naphthyl,
(3) pyridyl,
(4) furyl,
(5) thienyl,
(6) benzofuryl, and
(7) benzothienyl;

(i) C₁₋₆ alkanoyl C₁₋₆ alkyl;

(j) C₁₋₆ alkanoyloxy C₁₋₆ alkyl;

the above groups (a)- (j) can be unsubstituted or substituted with one or more radicals selected from a group consisting of C₁₋₆ alkyl, hydroxy, C₁₋₆ alkoxy, halo, nitro, mercapto, amino, N-C₁₋₆ alkyl or N,N-diC₁₋₆ alkylamino, cyano, carboxy, sulfoamino, carbamoyl, carbamoyloxy, sulfonyl, sulfinyl, sulfamoyl, azido, carboxamido and N-C₁₋₆ alkyl or N,N-diC₁₋₆ alkyl carboxamido;

B₃ is B₁ or hydrogen; or

B₂ and B₃ are joined such that together with the nitrogen to which they are attached there is formed a ring of 5 to 7 atoms having one nitrogen atom and optionally one oxygen atom, said ring being optionally substituted with a carboxyl; and Q is

- (1) hydrogen;
(2) C₁₋₆ alkyl;
(3) halo C₁₋₆ alkyl;
(4) hydroxy C₁₋₆ alkyl;
(5) methylene, C₁₋₆ alkylmethylene phenyl methylene, phenylthiomethylene, phenylsulfinylmethylene or phenylsulfonyl methylene wherein phenyl or alkyl can be unsubstituted or substituted as previously defined;

- (6) C₁₋₆ alkoxy C₁₋₆ alkyl;
(7) benzyl;
(8) phenylthio C₁₋₆ alkyl, phenylsulfinyl
C₁₋₆ alkyl
or phenylsulfonyl C₁₋₆ alkyl;
(9) phenoxy
C₁₋₆ alkyl; or
(10) phenylamino C₁₋₆ alkyl.

2. A pharmaceutical composition according to Claim 1 wherein

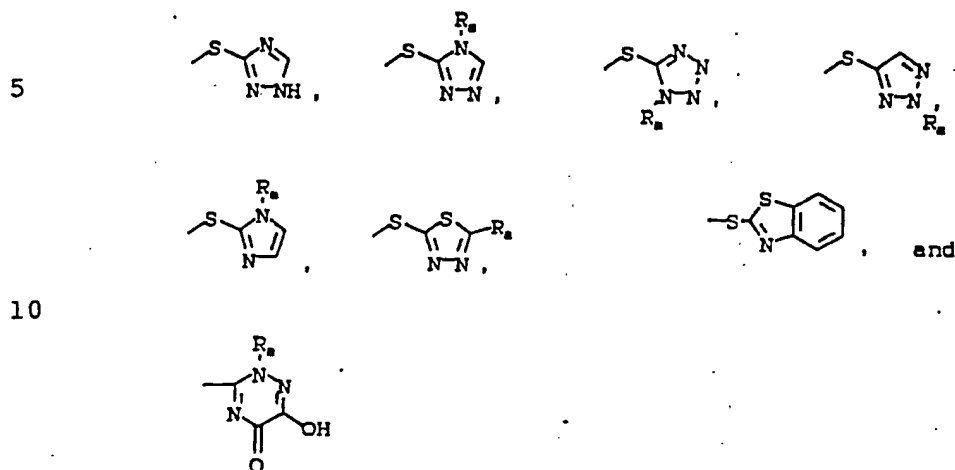
R is hydrogen, chlorine or fluorine;

M is:

- (1) trifluoromethyl;
(2) chloro or fluoro;
(3) -COOH;
(4) -CHO; or
(5) -CH₂A wherein A represents
(a) hydrogen;
(b) halo;
(c) ZR₅ wherein Z is oxygen or sulfur and
R₅ is
(1) straight or branched chain C₁₋₆
alkyl;
(2) H;
(3) straight or branched chain C₂₋₆
alkenyl; or
(4) straight or branched chain C₁₋₆
alkynyl;

- (d) acyloxy wherein acyl is C₂₋₆ alkanoyl, phenylcarbonyl, benzyloxycarbonyl, succinoyl, carbamoyl, N-C₁₋₆alkyl or N,N-diC₁₋₆alkyl carbamoyl, thiocarbamoyl, or N-C₁₋₆alkyl or N,N-diC₁₋₆alkyl- thiocarbamoyl;
- (e) phenoxy;
- (f) benzyloxy;
- (g) phenylthio or benzylthio;
- (h) acylthio wherein the acyl group is as previously defined;
- (i)
$$\begin{array}{c} \text{--S--C--OR}_a \\ \parallel \\ \text{S} \end{array} \quad \text{or} \quad \begin{array}{c} \text{--S--C--OR}_a \\ \parallel \\ \text{O} \end{array}$$
 wherein R_a represents C₁₋₆ alkyl, phenyl or CH₂COOH;
- (j) a substituted or unsubstituted amino or amido group selected from a group consisting of amino, N-C₁₋₆ alkylamino, N,N-diC₁₋₆ alkylamino;
- (k) R_a-SO- ; or
- (l) R_a-SO₂-;

(m) a substituent selected from the group consisting of



the above groups (a) to (1) can be unsubstituted or substituted with one or more radicals selected from a group consisting of C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, cyano, carboxy, carbamoyl, azido, sulfo, amino, C₁₋₆ alkylamino, diC₁₋₆ alkylamino, halo C₁₋₆ alkyl, N-C₁₋₆ alkyl-guanidino, guanido C₁₋₆ alkyl, sulfomyl and N-C₁₋₆ alkyl-sulfamyl;

(6) -CH=CH-R_a;

R₁ is

(a) hydrogen;

25 (b) hydroxy;

(c) OR'₁ wherein R'₁ is a hydrocarbyl group selected from a group consisting of straight or branched chain C₁₋₆ alkyl, C₃₋₆ alkenyl, C₃₋₆ alkynyl, phenyl and benzyl, these hydrocarbyl groups may be
30 unsubstituted or substituted with one or more radicals which is hydroxy, halo, nitro, amino, carboxy or thio;

(d) $-SR'_1$ wherein R'_1 is as previously defined;

(e) a hydrocarbyl group selected from a group consisting of

- 5
- (1) straight or branched C_{1-6} alkyl;
 - (2) phenyl C_{1-6} alkyl;
 - (3) phenyl;

the above groups (1) to (3) can be unsubstituted or substituted with one or more radicals selected from a group consisting of halo, hydroxy, C_{1-6} alkoxy, amino, nitro, sulfonyl, sulfamoyl, acyloxy, carbamoyloxy, carboxy, carboxamido and $N-C_{1-6}$ alkyl or $N,N-C_{1-6}$ alkylcarboxamido;

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(f) $\overset{\overset{X}{\parallel}}{C}-R''$ wherein X is oxygen or sulfur and R'' is

- (1) C_{1-6} alkyl;
 - (2) phenyl;
 - (3) benzyl;
 - (4) benzyloxy;
 - (5) C_{1-6} alkyloxy; or
 - (6) phenoxy;
- 20

(g) halo;

25

(h) $\overset{\overset{O}{\parallel}}{O}-C-CH_3$

(i) $-SO_2R_2$ where R_2 is C_{1-6} alkyl, halo C_{1-6} alkyl, phenyl, or benzyl which can be unsubstituted or substituted as previously defined;

30

B is OB_1 or NB_2B_3 wherein B_1 and B_2 independently are:

- (a) straight or branched chain alkyl having from 1 to 12 carbon atoms;

- (b) aryl having from 6 to 10 carbon atoms;
(c) cycloalkyl having from 3 to 8 carbon atoms;
(d) alkenyl having from 2 to 12 carbon atoms;
(e) alkynyl having from 2 to 12 carbon atoms;
5 (f) alkoxy alkyl having from 2 to 12 carbon atoms;
(g) aralkyl, alkaryl, aralkenyl, aralkynyl, alkenylaryl or alkynylaryl wherein alkyl, alkenyl and alkynyl are as previously defined, and the aryl group is selected from
10 the group consisting of
(1) phenyl,
(2) naphthyl,
(3) pyridyl,
(4) furyl,
15 (5) thienyl,
(6) benzofuryl, and
(7) benzothienyl;
(h) C₁₋₆ alkanoyl C₁₋₆ alkyl;
(i) C₁₋₆ alkanoyloxy C₁₋₆ alkyl;
20 the above groups (a)-(i) can be unsubstituted or substituted with one or more radicals selected from a group consisting of C₁₋₆ alkyl, hydroxy, C₁₋₆ alkoxy, halo, nitro, mercapto, amino, N-C₁₋₆ alkyl or
25 N,N-diC₁₋₆ alkylamino, cyano, carboxy, sulfoamino, carbamoyl, carbamoyloxy, sulfonyl, sulfinyl, sulfamoyl, azido, carboxamido and N-C₁₋₆ alkyl or N,N-diC₁₋₆ alkyl carboxamido;
B₃ is B₁ or hydrogen; or

B₂ and B₃ are joined such that together with the nitrogen to which they are attached there is formed a ring of 5 to 7 atoms having one nitrogen atom and optionally one oxygen atom, said ring being optionally substituted with a carboxyl; and
5 Q is

- (1) hydrogen;
- (2) C₁₋₆ alkyl;
- (3) halo C₁₋₆ alkyl;
- (4) methylene, C₁₋₆ alkylmethylene phenyl
10 methylene, phenylthiomethylene, phenylsulfinylmethylene or phenylsulfonyl methylene wherein phenyl or alkyl can be unsubstituted or substituted as previously defined;
- (5) C₁₋₆ alkoxy C₁₋₆ alkyl;
- (6) benzyl;
- (7) phenylthio C₁₋₆ alkyl, phenylsulfinyl
15 C₁₋₆ alkyl or phenylsulfonyl C₁₋₆ alkyl;
- (8) phenoxy C₁₋₆ alkyl; or
- (9) phenylamino C₁₋₆ alkyl.

25 3. A pharmaceutical composition according to Claim 2 wherein

wherein M is:

- (1) trifluoromethyl;
 - (2) chloro or fluoro;
 - (3) -COOH;
 - (4) -CHO; or
- 30

(5) $-\text{CH}_2\text{A}$ wherein A represents

- (a) hydrogen;
- (b) halo;
- (c) ZR_5 wherein Z is oxygen or sulfur and R_5 is

(1) straight or branched chain C_{1-6} alkyl;

(2) H;

(3) straight or branched chain C_{2-6} alkenyl; or

(4) straight or branched chain C_{1-6} alkynyl;

(d) acyloxy wherein acyl is C_{2-6} alkanoyl, phenylcarbonyl, benzyloxycarbonyl, succinoyl, carbamoyl, N-C_{1-6} alkyl or N,N-diC_{1-6} alkyl carbamoyl, thiocarbamoyl, or N-C_{1-6} alkyl or N,N-diC_{1-6} alkyl- thiocarbamoyl;

(e) phenoxy;

(f) benzyloxy;

(g) phenylthio or benzylthio;

(h) acylthio wherein the acyl group is as previously defined;

(i) $-\text{S}-\underset{\text{S}}{\underset{||}{\text{C}}}-\text{OR}_a$ or $-\text{S}-\underset{\text{O}}{\underset{||}{\text{C}}}-\text{OR}_a$

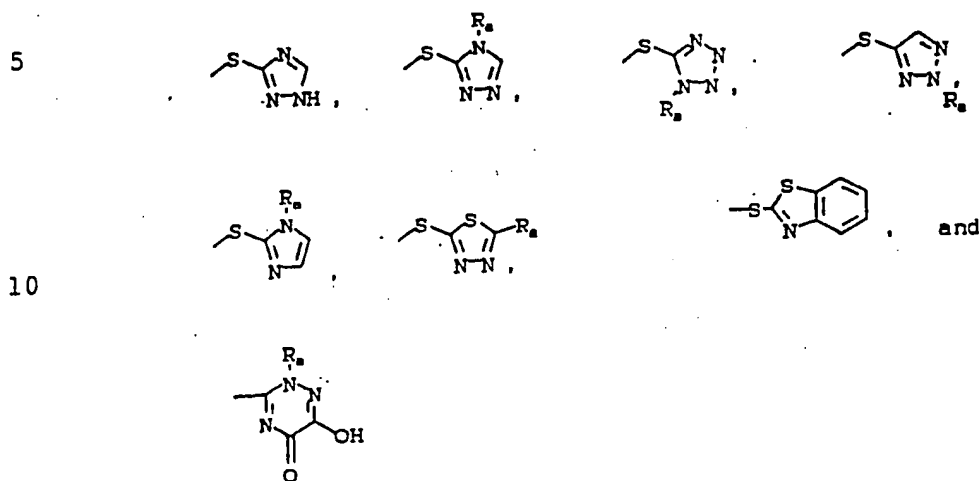
wherein R_a represents

C_{1-6} alkyl, phenyl or CH_2COOH_2 ;

(j) a substituted or unsubstituted amino or amido group selected from a group consisting of amino, N-C_{1-6} alkylamino, N,N-diC_{1-6} alkylamino;

(k) $\text{R}_a-\text{SO}-$; or

- (1) R_a-SO_2- ;
 (m) a substituent selected from the group consisting of



the above groups (a) to (1) can be unsubstituted or substituted with one or more radicals selected from a group consisting of C_{1-6} alkyl, C_{1-6} alkoxy, halo, cyano, carboxy, carbamoyl, azido, sulfo, amino, C_{1-6} alkylamino, diC_{1-6} alkylamino, halo C_{1-6} alkyl, $N-C_{1-6}$ alkyl-quanidino, quanido C_{1-6} alkyl, sulfomyl and $N-C_{1-6}$ alkyl-sulfamyl;

- (6) $-CH=CH-R_a$;

R_1 is

- (a) hydroxy;
 (b) OR'_1 wherein R'_1 is a hydrocarbyl group selected from a group consisting of straight or branched chain C_{1-6} alkyl, C_{3-6} alkenyl, C_{3-6} alkynyl, phenyl and benzyl, these hydrocarbyl groups may be unsubstituted or substituted with one or more radicals which is hydroxy, halo, nitro, amino, carboxy or thio;

(c) $-O-CO-CH_3$;

(d) straight or branched chain C_{1-4} alkyl;

B is OB_1 or NB_2B_3 wherein B_1 and B_2 independently are:

(a) straight or branched chain alkyl having from 1 to 6 carbon atoms;

(b) alkenyl having from 2 to 6 carbon atoms;

(c) alkoxy alkyl having from 2 to 6 carbon atoms;

(d) phenyl C_{1-6} alkyl or C_{1-6} alkyl phenyl;

(e) C_{1-6} alkenyl C_{1-6} alkyl;

(f) C_{1-6} alkanoyl C_{1-6} alkyl;

(g) C_{1-6} alkanoyloxy C_{1-6} alkyl;

the above groups (a)-(g) can be unsubstituted or substituted with one or more radicals selected from a group consisting of C_{1-6} alkyl, hydroxy, C_{1-6} alkoxy, halo, nitro, mercapto, amino, $N-C_{1-6}$ alkyl or N,N -di C_{1-6} alkylamino, cyano, carboxy, sulfoamino, carbamoyl, carbamoyloxy, sulfonyl, sulfinyl, sulfamoyl, azido, carboxamido and $N-C_{1-6}$ alkyl or N,N -di C_{1-6} alkyl carboxamido;

B_3 is B_1 or hydrogen; or

B_2 and B_3 are joined such that together with the nitrogen to which they are attached there is formed a ring of 5 to 7 atoms having one nitrogen atom and optionally one oxygen atom, said ring being optionally substituted with a carboxyl; and

Q is

(1) hydrogen;

(2) C_{1-6} alkyl;

(3) halo C_{1-6} alkyl;

(4) hydroxy C_{1-6} alkyl;

- 5 (5) methylene, C₁₋₆ alkylmethylene phenyl
methylene, phenylthiomethylene,
phenylsulfinylmethylene or
phenylsulfonyl methalene wherein phenyl
or alkyl can be unsubstituted or
substituted as previously defined;
- (6) C₁₋₆ alkoxy C₁₋₆ alkyl;
- (7) benzyl;
- 10 (8) phenylthio C₁₋₆ alkyl, phenylsulfinyl
C₁₋₆ alkyl
or phenylsulfonyl C₁₋₆ alkyl;
- (9) phenoxy
C₁₋₆ alkyl; or
- (10) phenylamino C₁₋₆ alkyl.

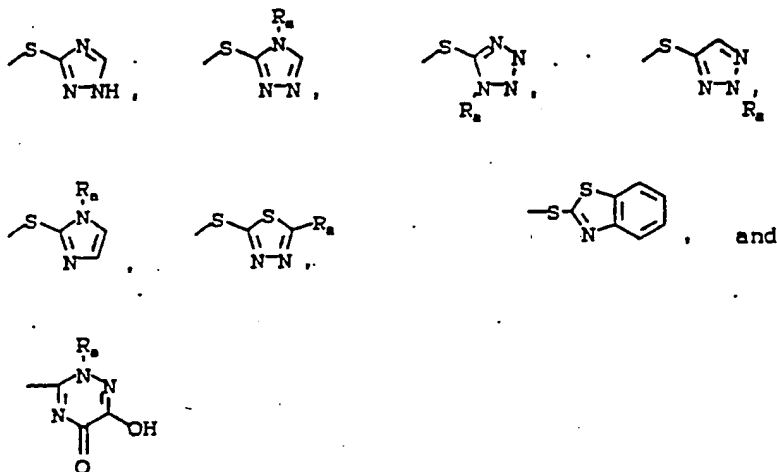
15 4. A pharmaceutical composition according
to Claim 3 wherein

wherein M is:

- 20 (1) chloro or fluoro; or
- (5) -CH₂A wherein A represents
- (a) hydrogen;
- (b) halo;
- (c) ZR₅ wherein Z is oxygen or sulfur and
R₅ is straight chain C₁₋₆ alkyl;
- 25 (d) acyloxy wherein acyl is C₂₋₆ alkanoyl,
phenylcarbonyl, benzyloxycarbonyl,
succinoyl, carbamoyl, N-C₁₋₆ alkyl or
N,N-diC₁₋₆ alkyl carbamoyl,
thiocarbamoyl, or N-C₁₋₆alkyl or
- 30 (e) phenylthio or benzylthio;

- (f) R_a-SO- wherein R_a is C_{1-6} alkyl or phenyl; or
 (g) R_a-SO_2- ;
 (h) a substituent selected from the group consisting of

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the above groups (a) to (g) can be unsubstituted or substituted with one or more radicals selected from a group consisting of C_{1-6} alkyl, C_{1-6} alkoxy, halo, cyano, carboxy, carbamoyl, azido, sulfo, amino, C_{1-6} alkylamino, di- C_{1-6} alkylamino, halo C_{1-6} alkyl, N- C_{1-6} alkyl-quanidino, guanido C_{1-6} alkyl, sulfomyl and N- C_{1-6} alkyl-sulfamyl;

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(6) $-CH=CH-R_a$;

R_1 is

- (a) OR'_1 wherein R'_1 is a hydrocarbyl group selected from a group consisting of straight or branched chain C_{1-6} alkyl, C_{3-6} alkenyl, C_{3-6} alkynyl, phenyl and benzyl, these hydrocarbyl groups may be unsubstituted or substituted with one or more radicals which is hydroxy, halo, nitro, amino, carboxy or thio;

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(b) $-O-CO-CH_3$;

(c) straight or branched chain C_{1-4} alkyl;

B is OB_1 or NB_2B_3 wherein B_1 and B_2 independently are:

(a) straight or branched chain alkyl having from 1 to 6 carbon atoms;

(b) alkenyl having from 2 to 6 carbon atoms;

(c) alkoxy alkyl having from 2 to 6 carbon atoms;

(d) phenyl C_{1-6} alkyl or C_{1-6} alkyl phenyl;

(e) C_{1-6} alkanoyloxy C_{1-6} alkyl;

the above groups (a)-(e) can be unsubstituted or substituted with one or more radicals selected from a group consisting of C_{1-6} alkyl, hydroxy, C_{1-6} alkoxy, amino, $N-C_{1-6}$ alkyl or N,N -di C_{1-6} alkylamino, carboxy;

B_3 is B_1 or hydrogen; or

B_2 and B_3 are joined such that together with the nitrogen to which they are attached there is formed a ring of 5 to 7 atoms having one nitrogen atom and optionally one oxygen atom, said ring being optionally substituted with a carboxyl; and Q is

(1) hydrogen;

(2) C_{1-6} alkyl;

(3) halo C_{1-6} alkyl;

(4) methylene, C_{1-6} alkylmethylene phenyl

methylene, phenylthiomethylene,

phenylsulfinylmethylene or

phenylsulfonyl methylene wherein phenyl

or alkyl can be unsubstituted or

substituted as previously defined;

(5) benzyl;

(6) phenylthio C_{1-6} alkyl, phenylsulfinyl

C_{1-6} alkyl

or phenylsulfonyl C_{1-6} alkyl; or

(7) phenoxy C₁₋₆ alkyl.

5. A pharmaceutical composition according to Claim 4 wherein

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R is hydrogen;

R₁ is (a) hydrogen,

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(b) -OR₁' wherein R₁' is a hydrocarbyl group selected from the group consisting of straight or branched chain C₁₋₄ alkyl, C₂₋₄ alkenyl, phenyl and benzyl groups,

(c) straight or branched C₁₋₄ alkyl, or

(d) bromo, chloro or fluoro;

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B is OB₁ or NB₂B₃ wherein B₁ and B₂ are each independently:

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- (1) straight or branched chain C₁₋₆ alkyl,
- (2) phenyl C₁₋₆ alkyl, or C₁₋₆ alkyl phenyl,
- (3) C₁₋₆ alkanoyloxy C₁₋₆ alkyl, or
- (4) carboxy C₁₋₆ alkyl;

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the above groups (1), (2) and (3) being unsubstituted or substituted with a radical selected from the group consisting of C₁₋₆ alkyl, hydroxy, and C₁₋₆ alkoxy;

B₃ is B₁ or hydrogen, or

B₂ and B₃ are joined such that together with the nitrogens to which they are attached there is formed a ring of 5 to 7 atoms having one nitrogen atom and optionally one oxygen atom, said ring being optionally substituted with a carboxyl; and

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Q is

- (1) hydrogen,
- (2) C₁₋₃ alkyl,
- (3) methylene,
- (4) benzyl, or
- (5) phenoxy C₁₋₃ alkyl.

6. A pharmaceutical composition according to Claim 5 wherein

R₁ is -OCH₃, chlorine or ethyl;

Q is hydrogen;

M is: (a) -CH₃,

(b) CH₂-OAc,

(c) chloro, or

(d) ((1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)thio)methyl;

B is OB₁ or NB₂B₃ wherein B₁ and B₂ are each independently;

(1) straight or branched chain C₁₋₆ alkyl,

(2) phenyl C₁₋₆ alkyl, or C₁₋₆ alkyl phenyl,

(3) C₁₋₆ alkanoyloxy C₁₋₆ alkyl, or

(4) carboxy C₁₋₆ alkyl;

the above groups (1), (2) and (3) being unsubstituted or substituted with a substituent selected from the group consisting of C₁₋₆ alkyl, hydroxy, carboxy and C₁₋₆ alkoxy;

B₃ is B₁ or hydrogen; or B₂ and B₃ may join together to form a substituted heterocyclic ring of 5 or 6 atoms, said ring having one nitrogen atom, or one nitrogen atom and one oxygen atom, wherein said substituent is hydrogen or carboxy.

7. A pharmaceutical composition according to Claim 6 wherein

R₁ is -OCH₃;

M is (a) -CH₂-OAc, or

5 (b) ((1,2,5,6-tetrahydro-2-methyl-5, 6-dioxo-1,2,4-triazin-3-yl)thio)methyl; and

B is NB₂B₃ wherein

10 (a) B₂ is methyl and B₃ is carboxymethyl, or
(b) B₂ and B₃ are joined together to form,
(1) substituted pyrrolidine, wherein the substituent is carboxy,
(2) morpholino, or
(3) piperidino.

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8. A pharmaceutical composition according to Claim 7 wherein the compound of Formula I is

- 20 (1) (6R-Cis)-Pyrrolidine, 1-((7-methoxy-8-oxo-3-(((1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)thio)methyl)-5-thia-1-azabicyclo (4.2.0)oct-2-en-2-yl)carbonyl)-, 5,5-dioxide;
- 25 (2) 1,1-Dimethyl ethyl 3-((acetyloxy)methyl)-7- α -methoxy-8-oxo-5-thia-1-azabicyclo (4.2.0)oct-2-ene-2-carboxylate 5,5-dioxide;
- 30 (3) (6R-Cis)-L-proline, 1-((7-methoxy-8-oxo-3-((1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)thio)methyl)-5-thia-1-azabicyclo (4.2.0)oct-2-en-2-yl)carbonyl)-, 5,5-dioxide;

- (4) (6R-Cis)-L-proline, 1-((3-((acetyloxy)-methyl)-7-methoxy-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-yl)carbonyl)-, S,S-dioxide;
- 5 (5) (6R-Cis)-Morpholine, 4-((3-((acetyloxy)-methyl)-7-methoxy-8-oxo-5-thia-1-azabicyclo (4.2.0)oct-2-ene-2-yl)carbonyl)-, 5,5-dioxide;
- 10 (6) (4-carboxyphenyl)methyl-3-((acetyloxy)-methyl)-7 α -methoxy-8-oxo-5-thia-1-azabicyclo (4.2.0)oct-2-ene-2-carboxylate 5,5-dioxide; or
- 15 (7) 3-acetyloxymethyl-7 α -methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-(2-(S)-carboxypyrrolidinecarboxamide)-5,5-dioxide.

9. A pharmaceutical composition according to any of claims 1, 4, 8 or 9 for use in treating leukemia.

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10. A pharmaceutical composition according to any of Claims 1, 4, 8 or 9 for use in inhibiting proteinase 3/myeloblastin.

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Patents Act 1977
Examiner's report to the Comptroller under
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Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: CAS ONLINE

Date of Search

Documents considered relevant following a search in respect of claims 1-10

| Category (see over) | Identity of document and relevant passages | Relevant to claim(s) |
|------------------------|--|-------------------------|
| A | EP 0267723 A2 (MERCK) see for example formula I | 1 at least |

| Category | Identity of document and relevant passages | Relevant to claim(s) |
|----------|--|----------------------|
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